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Fine structure of the regional differentiation of the ductus ejaculatorius simplex (EJ1, EJ2, EJ3) along with the role of their secretions in sperm activation and motility in two Noctuid species, *Heliothis armigera* (Hübner) and *Spodoptera litura* (Fabricius) (Lepidoptera).

I. Fine structure and function of simplex 1 (EJ1)

G AMALDOSS

Biology Department, Fu Jen University, Hsinchuang 24205, Taipei, Taiwan, Republic of China

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Abstract. The common unpaired ejaculatory duct or simplex of male reproductive tract of *Heliothis armigera* and *Spodoptera litura*, on the basis of morphology and anatomy, are broadly distinguished into EJ1, EJ2 and EJ3 regions. The proximal region (EJ1) of common duct to the ductus ejaculatorius duplex characteristically contains transparent elastic gelatinous secretion which is capable of sperm activation *in vitro*. The immotile spermatozoa within the duplex are induced-*in vitro* activated by diluting the same to 0.15 M in HEPES-KOH buffer, maintaining pH at 7. The activation is facilitated by the addition on 10 mg/ml bovine serum albumin and activation is triggered by an addition of a small fraction of activator substance taken from EJ2. EJ1 of both species exhibit only a single layered epithelium. Merocrine and apocrine are the mode of secretion occurring in *Heliothis armigera* while merocrine is the only mode of secretion occurring in EJ1 of *Spodoptera litura*.

Keywords. Apocrine; merocrine; simplex; sperm activation.

1. Introduction

Basic histological studies of the internal male reproductive tract of Lepidoptera are many while ultra studies of the same have been restricted to a few species (Riemann and Thorson 1976a, b, 1979a, b; Lai-Fook 1982a-e; Amaldoss 1987, 1988). The role of secretions of common ejaculatory duct of Lepidoptera with regard to sperm activation and motility has been studied scantily (Omura 1936, 1938; Shepherd 1974a, b, 1975). The present study is prompted by the above studies and attempts to describe the fine structure with relation to their sections which is responsible for activation of sperm during ejaculation in *Heliothis armigera* and *Spodoptera litura*.

2. Materials and methods

2.1 Animals

Pupae of *H. armigera* and *S. litura* were obtained from Entomology Laboratories of AVRDC Tainan, Taiwan and were maintained in Long Light Co., Growth Chamber at 27-29°C and were entrained in a 12:12 light and dark environment. The RH was maintained at 60±5%. The adult moths were fed with 10% honey

solution. The moths in this experiment were 2-days old virgin males and 4-days old mated males.

2.2 *Light and electron microscopy*

The ductus ejaculatorius simplex was dissected out from the male reproductive tract in the Weevers (1966) lepidopteran saline and fixed in Zenker's solution for 3 h and then dehydrated in alcohol series. The tissues then were embedded in paraplast, 5 mm sections were cut and stained in Meyers hematoxylin and counterstained with eosin and observed under a phase contrast microscope. Then photographs were taken in the microscope. Simplex of virgin and mated males were fixed using 2% glutaraldehyde with 0.1 M phosphate buffer (pH 7.4) for 3 h at 4°C and then post fixed in 1% osmium tetroxide (OsO₄) in the same condition. After dehydration in acetone series tissues were embedded in Spur Epon 812. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and viewed under JEM100 at 45 kV.

2.3 *Preparation of sperm activator and sperm activation assay*

Virgin male moths (3 or 4-days old) were cold anaesthetized for 2 min and were dissected out in Weevers (1966) lepidopteran saline and the rest of the procedure was as that of Shepherd (1974a) to prepare the semen. Activator secretion was prepared from the common ejaculatory duct as that of Shepherd (1974a) and was separated into 3 divisions viz. EJ1, EJ2 and EJ3. Assays of sperm activating capacity was done as that of Shepherd (1974a). Tissues other than common ejaculatory duct of *H. armigera* and *S. litura* were homogenized in 80 µl as described by Shepherd (1974a) and tested for the activating capacity.

3. Results

Ductus ejaculatorius simplex 1 or EJ1 of *H. armigera* measures 4.3 cm in length while the same in *S. litura* measures 3 cm. EJ1 is the first non cuticular region of the common ejaculatory duct which contains transparent gelly like secretion both in *H. armigera* and *S. litura* and is the longest of 3 subdivisions of the common ejaculatory duct. EJ1 commences at the union of ductus ejaculatorius duplex in both the species. EJ1 consists of a single layered secretory epithelium with low columnar cells in both the species (figures 25, 26). The nuclei are dark, rounded and located in the median line. The epithelial cells do not close off the lumen as in the case of duplex in *S. litura* (Amaldoss 1987). The lumen is filled with globules type of secretory materials.

3.1 *Fine structure*

At the ultrastructural level, EJ1 of the common non cuticular ejaculatory duct reveals a single secretory epithelial cell type. As the EJ1 is non cuticular it lacks an intima for the epithelium. The epithelium and the muscle layers are intimately

associated with each other (figure 1). EJ1 of both species have the same manner of muscle arrangements surrounding the epithelium and prominent basement membrane. There is an outer longitudinal muscle layer and an inner circular muscle layer surrounding the basement membrane (figures 1, 6). The outer longitudinal muscle layers are thick and robust while the inner circular muscle layers are thin and scanty. The muscle layers are well supplied with tracheoles and nerve endings containing synaptic and dense cored vesicles. The muscle layers also contain elongated nuclei with dark and dense chromatin. The circular muscle layer of *S. litura* is found to be thicker than that of *H. armigera* while the basement membrane of *H. armigera* is more pronounced than that of *S. litura* (figures 1, 6, 20, 21). The distinguishing features of the epithelial cells are the occurrence of only merocrine secretory cells in *S. litura* and the occurrence of both apocrine and merocrine secretory cells in *H. armigera*. Merocrine type of secretory cells in EJ1 of *S. litura* secretes the gelly like substance to be accumulated at the free cell surface and released into the lumen, without the cytoplasmic loss. Apocrine secretory cells in EJ1 of *H. armigera* secretes the gelly substance along with cytoplasmic loss besides secreting merocrine secretion into the lumen.

The merocrine secretory cell appears to release its secretions by means of well pronounced micro villate surface (figure 13). The apical regions of merocrine secretory cell has numerous cell foldings for synthesis of materials and accumulation of secretory materials only to be transported through microvilli surface by means of exocytosis (figures 13, 14). The secretions are released into the lumen presumably by exocytosis. Micro tubules in the microvilli are well pronounced and resolved well in the electron microscopy (figure 13). The merocrine cells have elongated oval nuclei which are apically located (figures 3, 15). The cytoplasm of the merocrine cell is electron luscent. Rough endoplasmic reticulum is distended. Mitochondria and Golgi complexes are evenly distributed throughout the cell (figures 8, 9, 11). Microvilli are spatially arranged. Homogeneous electron light materials and electron dense particles are also seen. There is a group of secretory granules that are found in the merocrine cell; they are well defined, large, oval shaped and are membrane bound (figures 7, 12, 14, 15). Most of them contain electron dense materials at the centre of their body. The apocrine secretory cell appears to release its apical section along with secretory materials (figures 2-4, 10). This cell has an irregularly shaped nucleus located apically. The chromatin materials within the nucleus are sparsely distributed. The cytoplasm of apocrine secretory cell is denser than the cytoplasm of the merocrine type cell. There are numerous mitochondria at the apical region. Microtubules, smooth endoplasmic reticulum, electron dense secretory vesicles, electron light secretory vesicles and multivesicular bodies are abundant. Rough endoplasmic reticulum is more flat and sparse. The most conspicuous part of the apocrine cell is the free cell surface of apical region which extends deep into the lumen in the form of thin tongue like structures (figures 4, 5, 10). Apical membrane is thrown into extensive tight folds. The elongate apical projections (membranes) are typically fused in pairs like leaves and form complex multi layered whorls. In mated insects, they become part of the ejaculate and may take part in the form of the spermatophore (figures 4, 5, 10). The cytoplasm of apocrine cell consists of electron light secretory vesicles and membrane bound organelles particularly the mitochondria. The apocrine secretory materials occupy a larger proportion of the lumen than the merocrine type of secretory granules. These

cells are characterised by the presence of abundant rough endoplasmic reticulum, free ribosomes and Golgi complexes associated with dense cored particles. The free cell surface of the apocrine secretory cell exhibit irregularly shaped microvilli and secretory materials are released through the microvilli.

The cross section topography of EJ1 in *H. armigera* reveals a thick outer epithelial region, which presumably consists of muscle layers and basement membrane (figures 16, 17, 19). The epithelium appears to be a single layer of cells but with distinct basal and apical regions revealing thereby the merocrine and apocrine secretory cell types respectively (figure 17). The topography of the epithelium indicates that it consists of low columnar cells. The differing secretory cell types exhibit the type of materials they secrete which are expelled into the lumen.

The secretory granules that are produced by the merocrine type are hexagonal with granular rough surfaces (figure 18). The secretory epithelium of EJ1 in *S. litura* exhibits only merocrine secretory cell type. The secretory epithelium is characterised by the presence of abundant rough endoplasmic reticulum (figures 20–24). The secretory materials are accumulated at the free cell surface and are released into the lumen by breaking open the plasma membrane (figures 22, 30). There is no clear evidence of the occurrence of apocrine activity. The merocrine secretory cell has oval shaped nucleus which is basally located (figures 24, 27, 28). Golgi bodies and mitochondria are evenly distributed throughout the cytoplasm. Numerous free ribosomes and microtubules occur abundantly. The conspicuous feature of merocrine secretory cells is the presence of two groups of secretory granules or vesicles (figures 21, 29, 31–34). The secretory vesicles SV1, SV4, SV5 and SV6 comprise one distinct group with minor differences; they are either membrane bound or free, oval or spherical in shape. They are scattered in the cytoplasm. The membrane is curly in SV4 and SV5 granules and contain dense materials within, SV4 type has tooth like structures at the edges of the granules. The contents of this differ from those of SV4 and SV5—SV1 and SV6 are not membrane bound. SV1 also contains tooth like structures. Secretory granule SV2 is unique in being a multigranular vesicle, while SV3 is also unique in possessing double membrane and their contents are electron dense and light cored particles.

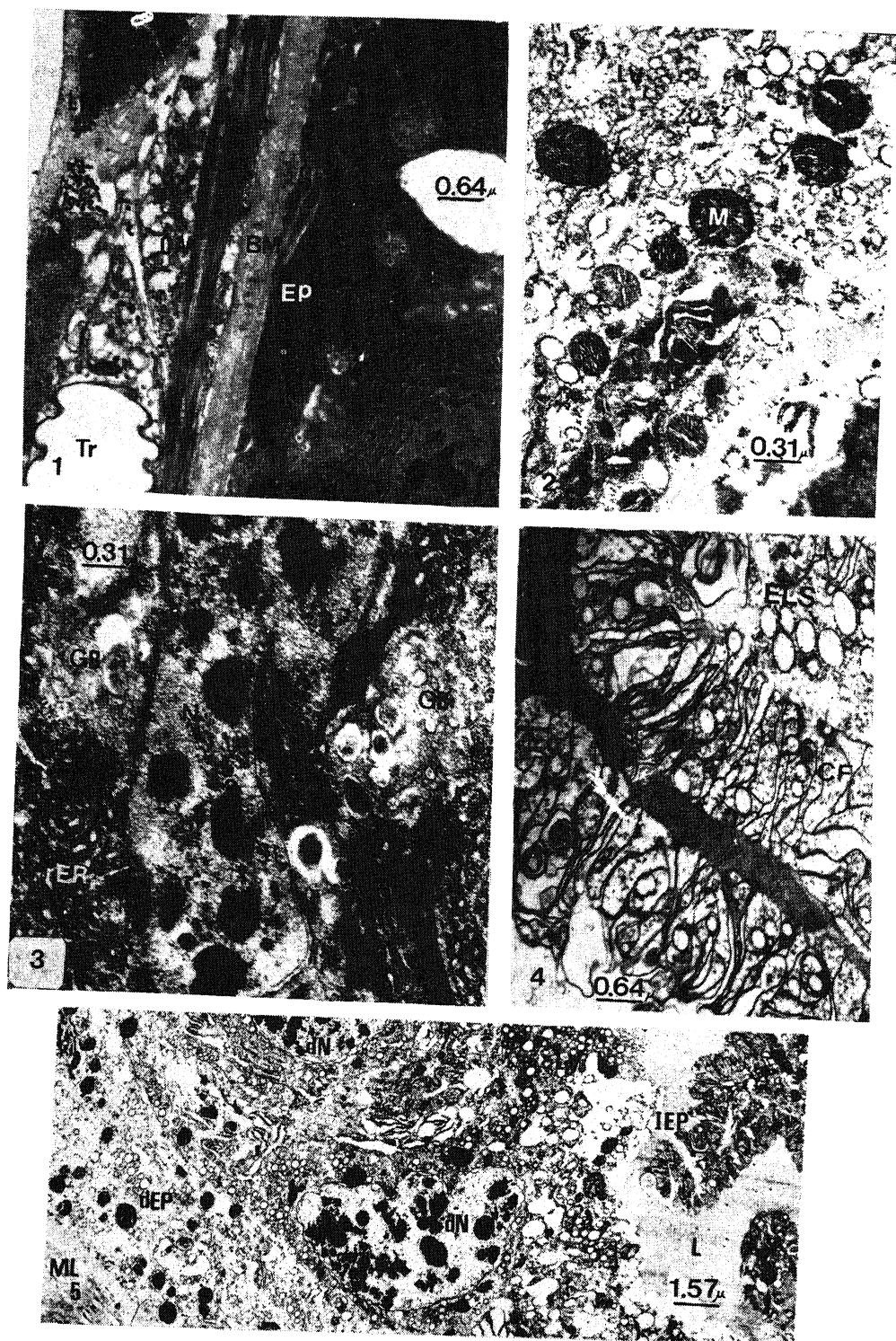
A second group of spherical and cylindrical secretory granules are bounded by a single membrane. They appear in clusters within the cytoplasm and are compartmentalised. The cytoplasm contains rER in lamellar form. The double nuclear membrane is well marked and the nucleus has more than 4 nucleoli. The chromatin materials are sparse and scattered. Golgi bodies appear to crowd around the nucleus. Tight junctions and desmosomes hold the neighbouring cell membranes together.

3.2 Localisation of sperm activation substances using elimination method

By elimination method EJ1 and EJ2 were found to contain activating capacity. Sperm from SV showed no activation while the same from duplex showed activation. Besides simplex, no other tissue exhibited any activation.

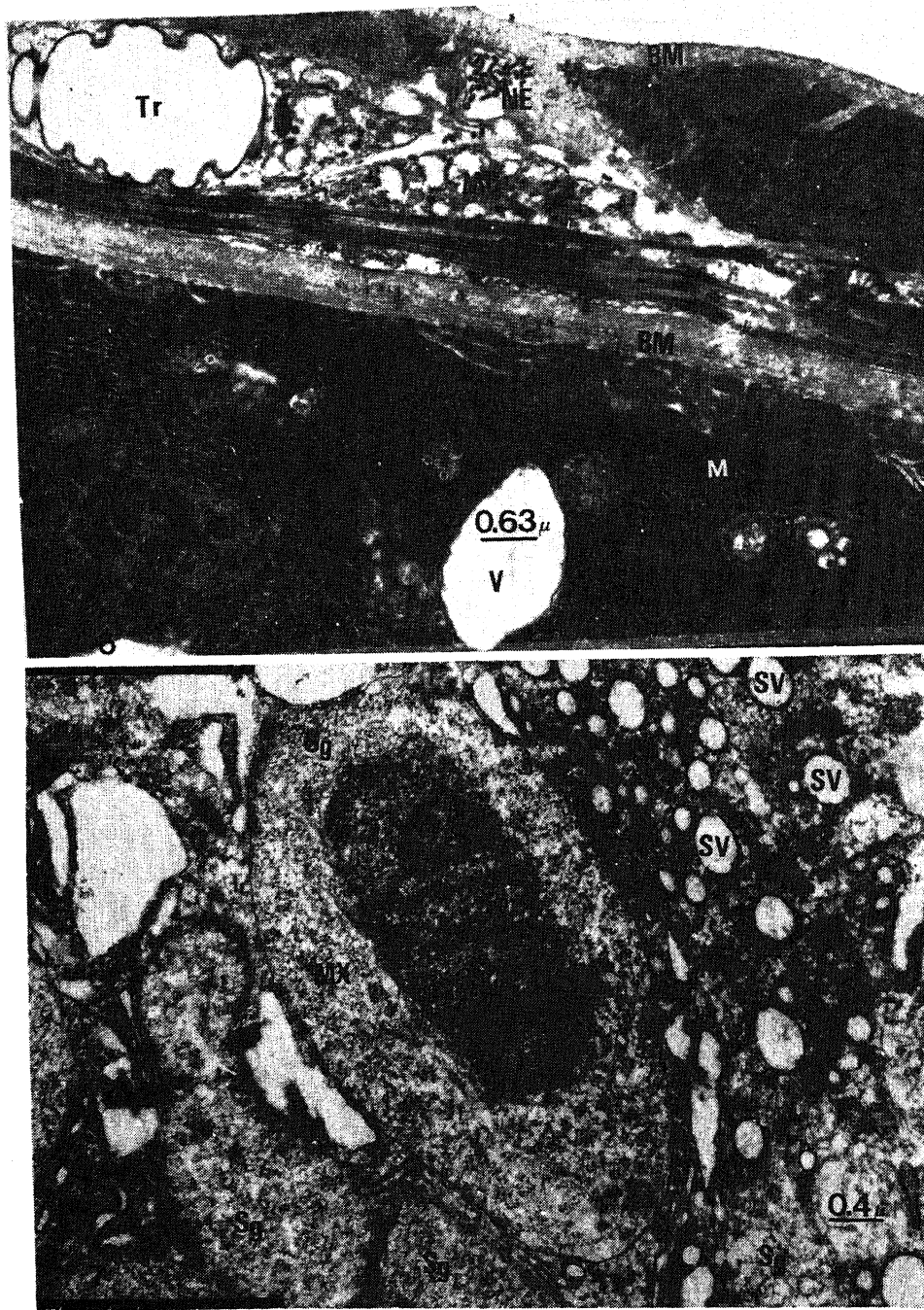
4. Discussion

The ductus ejaculatorius simplex is considered by most of the morphologists as the

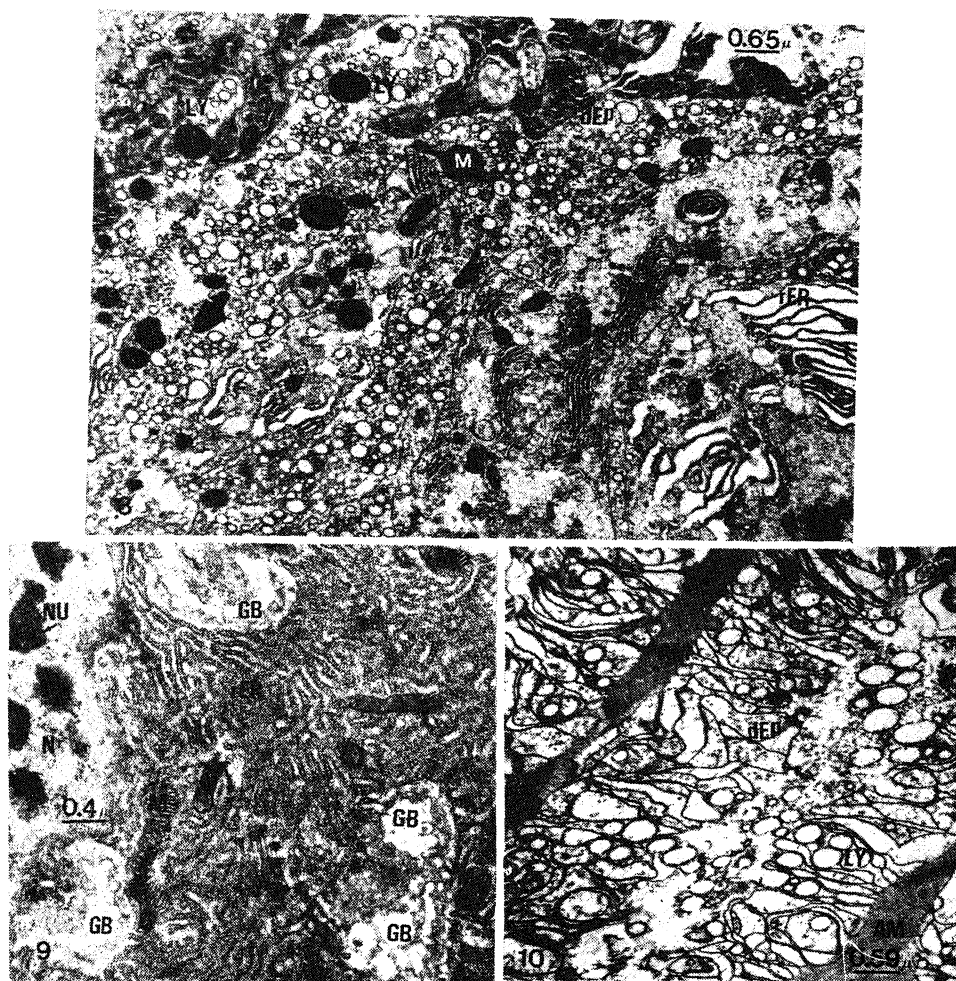


Figures 1-5. For caption, see p. 16.

entire duct leading from the endophallus to the union of the duplex gland (Callahan 1958). In *Heliothis zea*, Callahan (1958) distinguishes the cephalad portion of the simplex as the primary segment, and the caudal portion of the simplex as the

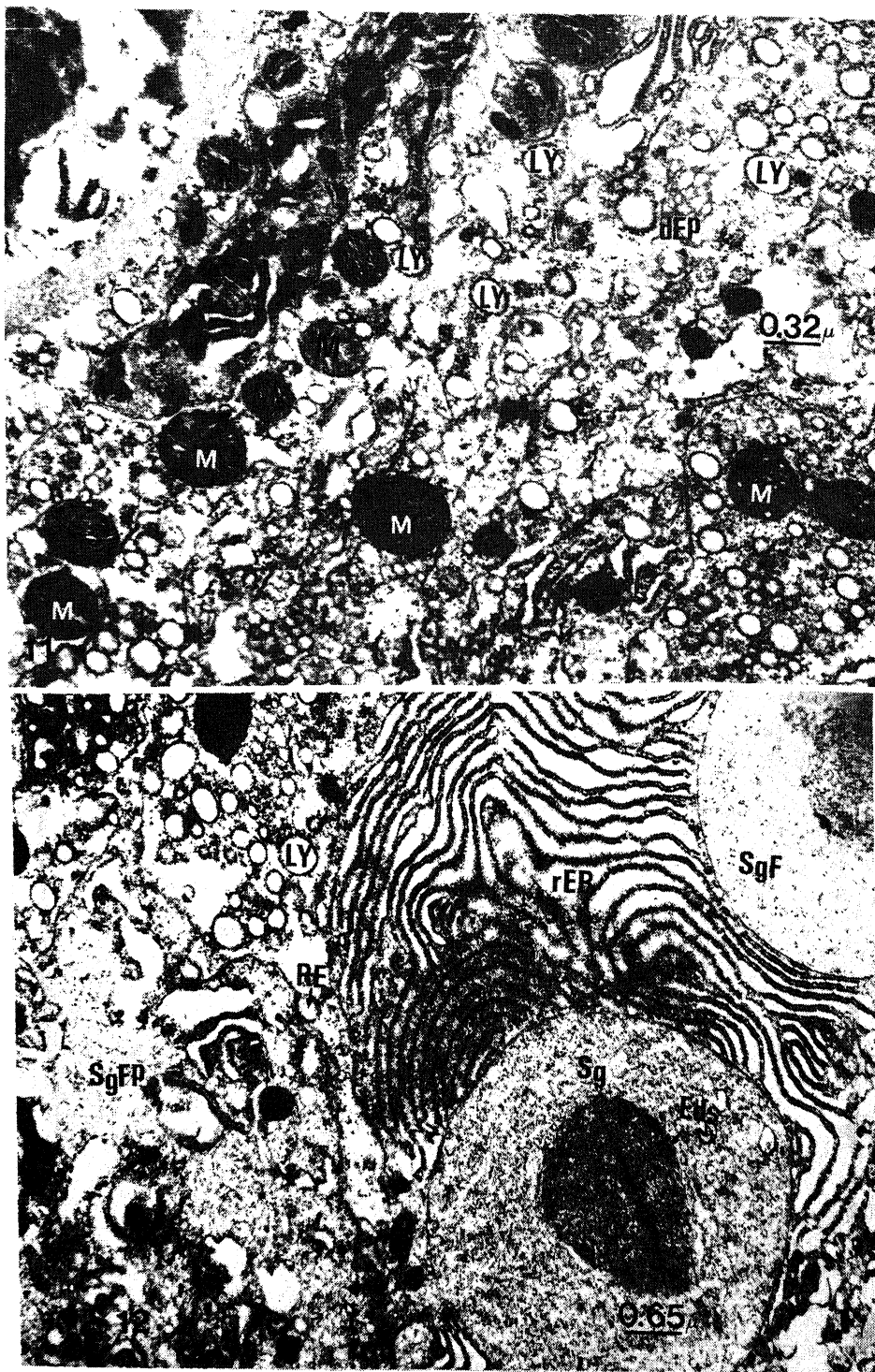


Figures 6-7. For caption, see p. 16.

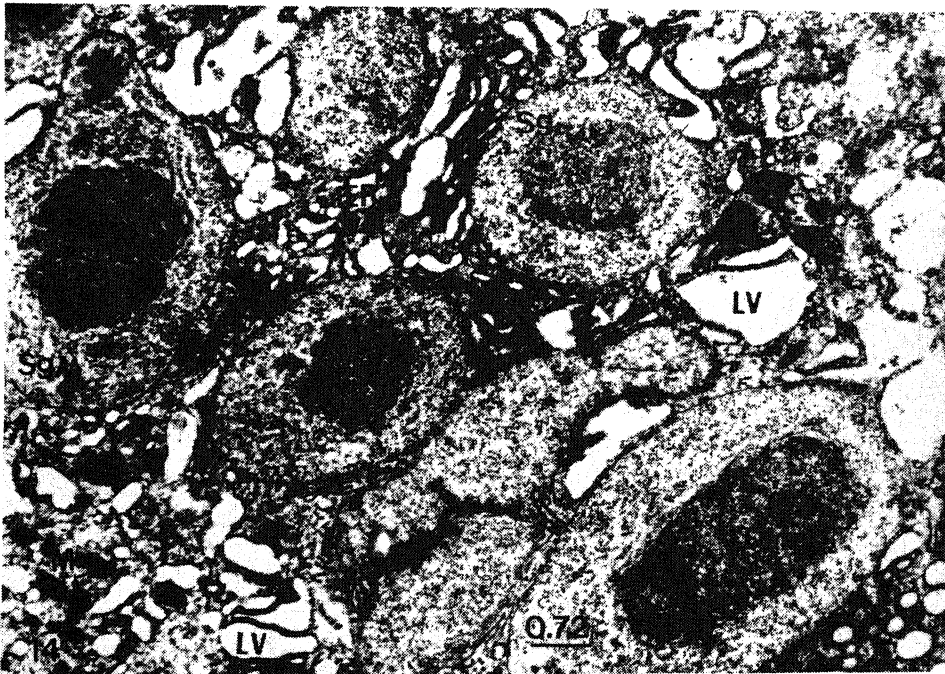
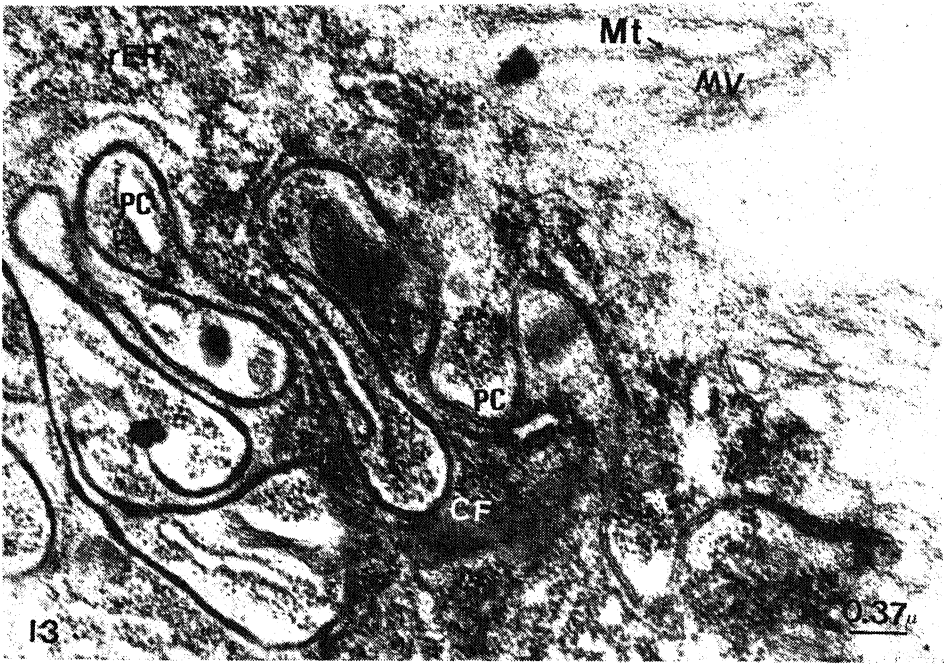


Figures 8-10. For caption, see p. 16.

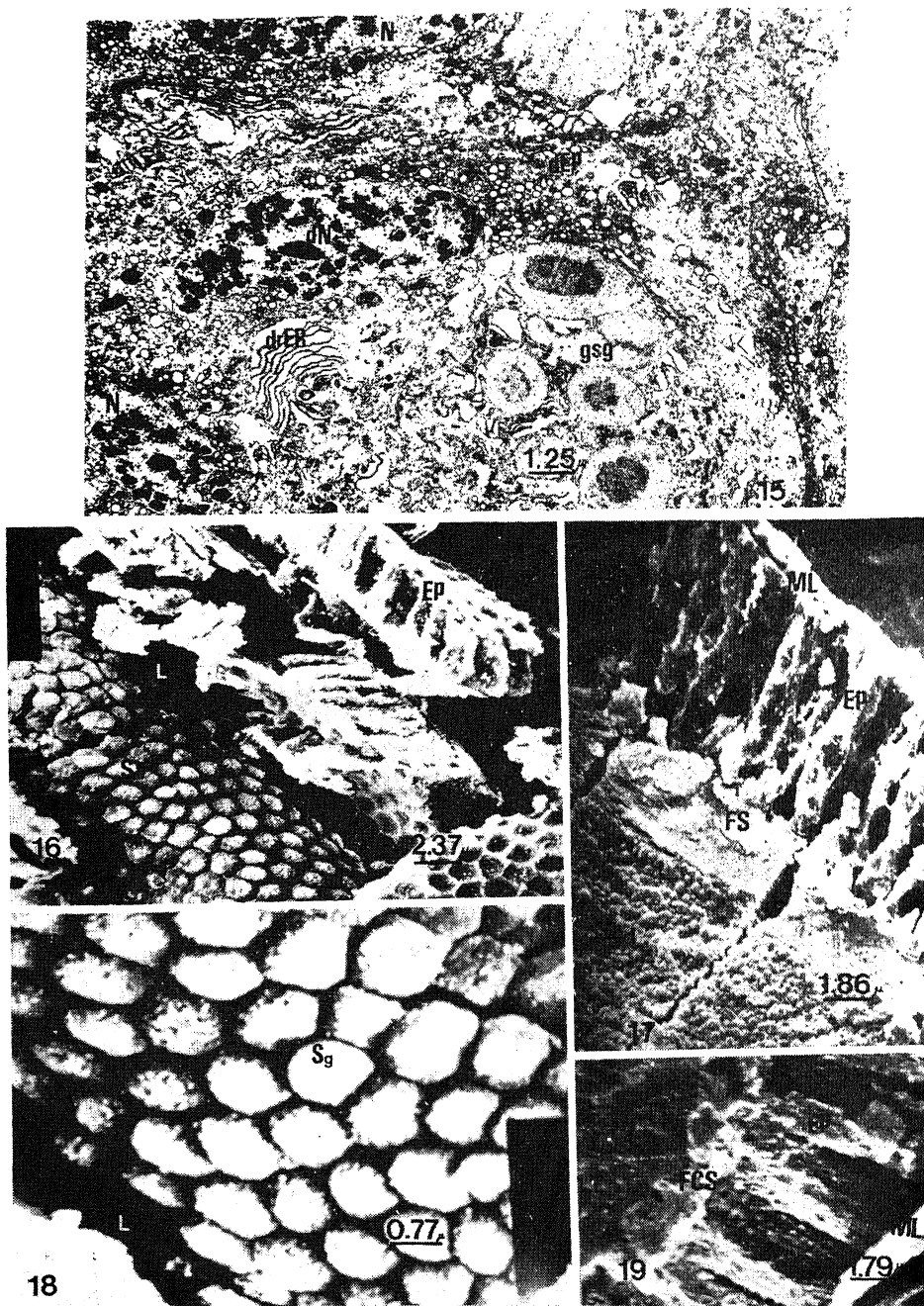
cuticular segment. In the present study, however, the simplex is considered as the duct leading from the union of the duplex gland to the part of the cuticular region, designated as EJ3. These divisions are made on the basis of morphological distinctions as well as their functional role in the secretion of the sperm activator substance. It is significant that EJ2 contains this activator substance in *S. litura* whereas EJ1 and EJ2 are jointly responsible for the activator substance in *H. armigera*. The cuticular simplex of these two species corresponds to the tightly looped caudal region of the simplex in *H. zea* (Callahan 1958), to the shoe-horn shaped part in *Pseudaletia unipuncta*, and to the curved half-moon shaped swelling in *Peridroma margaritosa* (Callahan and Chapin 1960). These differences are attributed to the function of the cuticular simplex in the formation of the frenum of the spermatophore (Callahan and Chapin 1960; Buntin and Pedigo 1983). It is also noteworthy that the width of the primary segment shows considerable variations depending upon the quantity of their contents.



Figures 11-12. For caption, see p. 16.

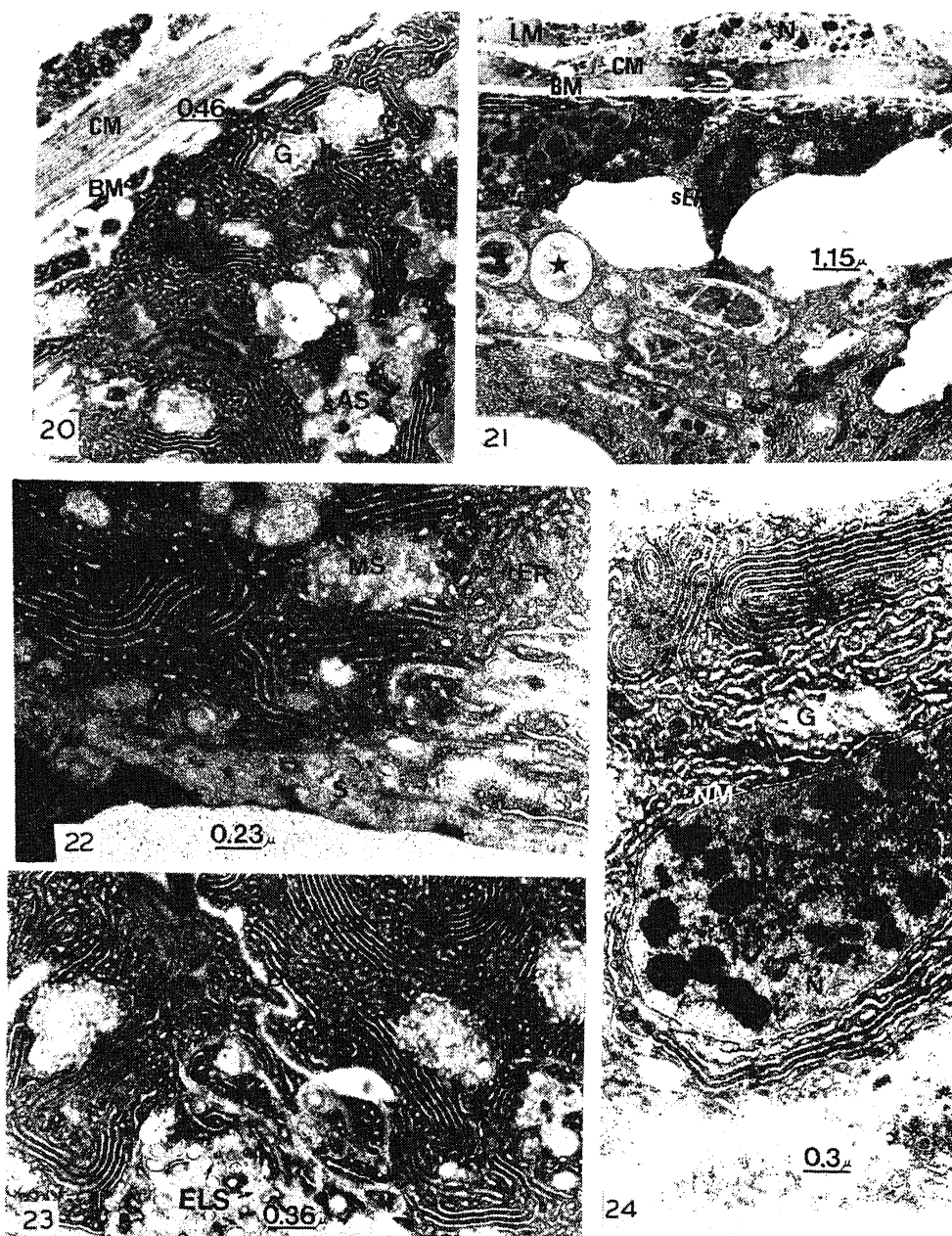


Figures 13-14. For caption, see p. 16.



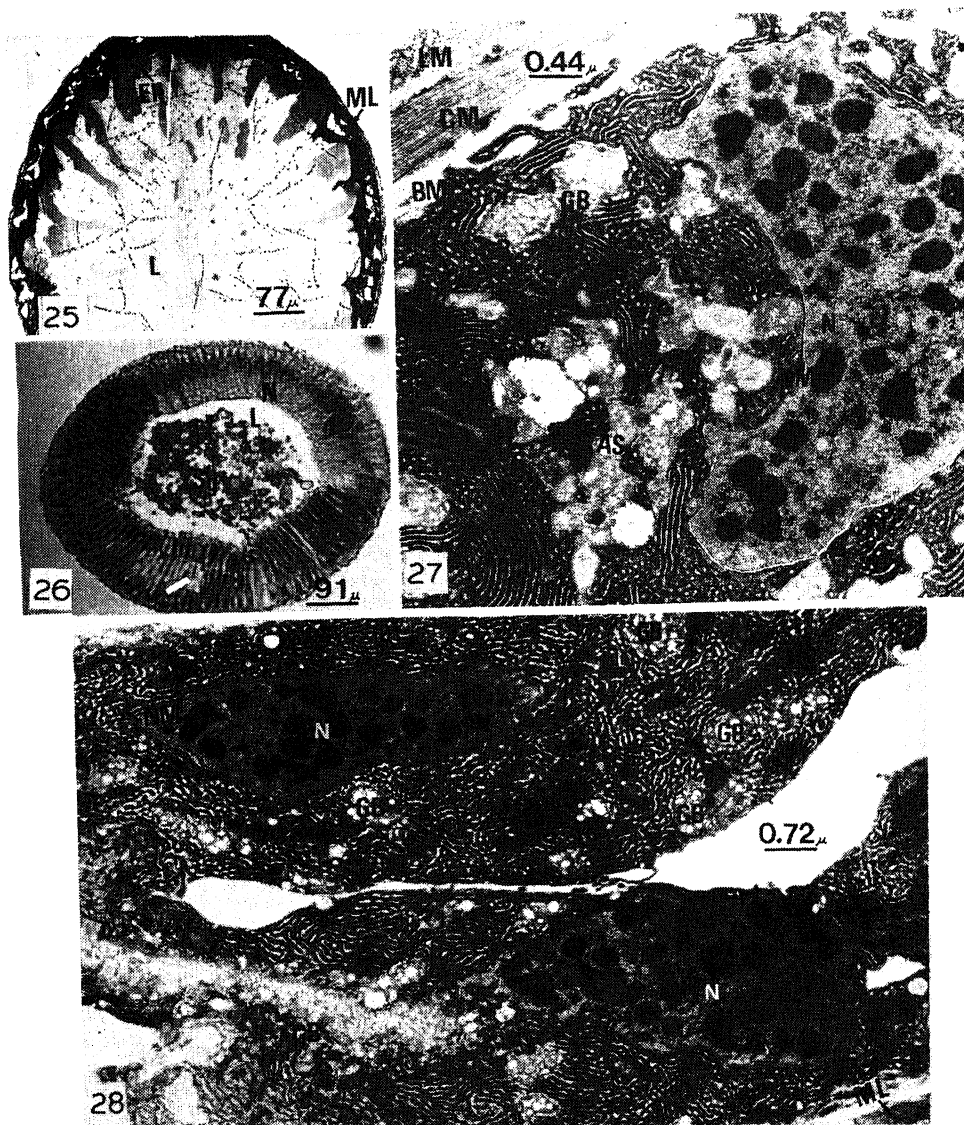
Figures 15–19. For caption, see p. 16.

The simplex of *S. litura* and *H. armigera*, as stated earlier, is divided into 3 segments, viz. EJ1, EJ2 and EJ3, on the basis of their functional morphology and their secretions responsible for sperm activation and sperm motility. The first two segments are non-cuticular while the third segment is cuticular in as much as it has



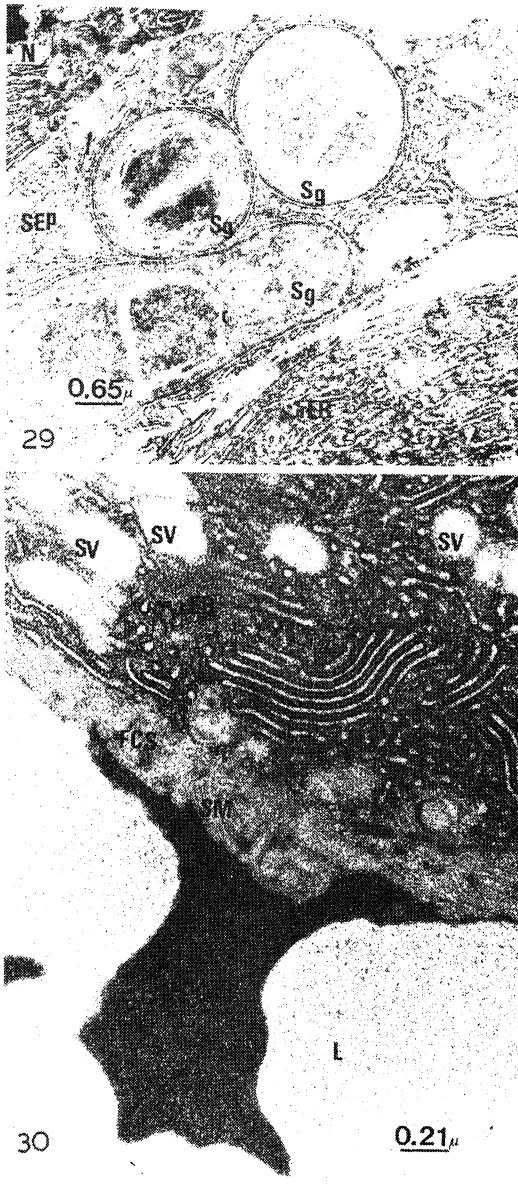
Figures 20-24. For caption, see p. 17.

a lining of intima. The 3 segments also differ in their relationships with regard to the secretory epithelium, basement membrane and muscle layer. EJ1 and EJ2 are not lined by an intima and their muscle layers are intimately associated with the epithelium as shown by the 'fusion' of their basement membranes, while in EJ3 the muscle layers are widely separated from the secretory epithelium, and this part



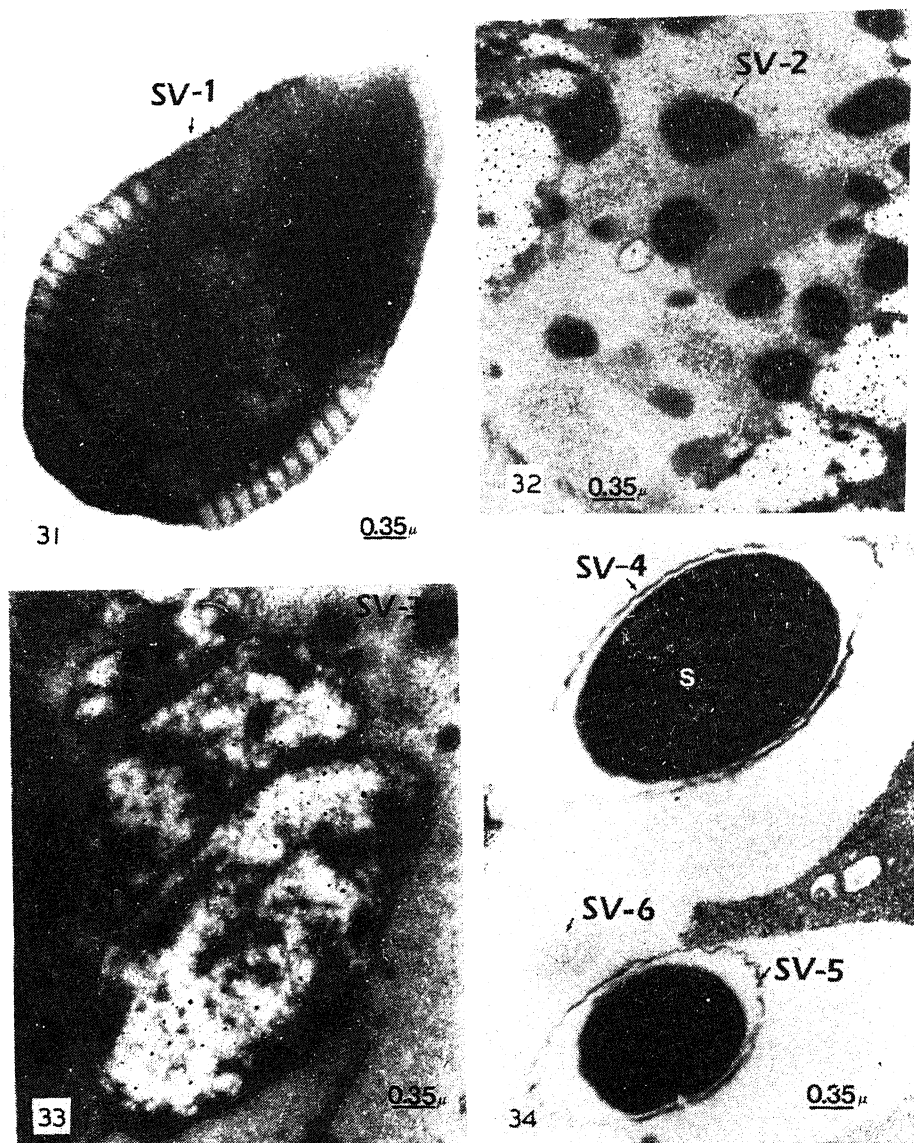
Figures 25-28. For caption, see p. 17.

corresponds to the 1st and 2nd portions of the ductus ejaculatorius simplex *Calpodex ethlius* reported by Lai-Fook (1982b), to the cuticular simplex of Callahan and Cascio (1963), to the ejaculatory duct of Musgrave (1937), Brits (1978) and Leclercq-Smekens (1974), and to the ejaculatory duct cum penis (Thibout 1971). The most notable feature in the arrangement of the muscle layers in the 3 EJ regions of *S. litura* and *H. armigera* is the uniformity, in having a thin, scant inner circular muscle layer, and a thick, robust outer longitudinal muscle layer, whereas in other species of Lepidoptera studied, there are differences in the wall of the simplex in the matter of the presence or absence of the muscle layers, and the relative thickness of the layers, when muscle layers occur. For instance, Musgrave (1937) reported t



Figures 29–30. For caption, see p. 17.

absence of muscle layers in the simplex in *Ephestia kuhniella*, except in the posterior terminal region. Again, Callahan and Cascio (1963) reported the presence of an inner longitudinal and an outer circular muscle layer in the anterior region of *H. zea*. Brits (1978) did not find any muscle layers in *Phthorimaea operculella*, while Riemann and Thorson (1979a) observed thin layers of muscles around atleast a portion of the simplex. In the arrangement of the muscle layers, *S. litura* and *H. armigera* approach that of *Calpodes ethlius* reported by Lai-Fook (1982c) very closely, with the circular layer of muscle always inner in position, thin and scant,



Figures 31-34. For caption, see p. 17.

while the longitudinal layer of muscle always outer in position and thick and robust. Further, as in *C. ethlius*, the two species studied here show an abundance of nerve endings and tracheae entering the muscle layers of the simplex throughout its length. The modes of secretion are also noteworthy in the region of the simplex. EJ1 of the simplex in *H. armigera* exhibits two types of secretion, viz. apocrine and merocrine, similar to the earlier reports of Riemann and Thorson (1979a) in *Ephestia* sp., and Lai-Fook (1982c) in *C. ethlius*. Musgrave (1937) recognised both apocrine and merocrine types of secretion in *Ephestia*. The EJ1 of *S. litura*, on the other hand, exhibits only the merocrine cell type.

The conditions necessary for sustaining the motility of Noctuid sperm are not

stringent as reported in Saturniid sperm (Shepherd 1974a) provided the total osmolarity is between 80 and 190 milliosmolar, pH between 5.8 and 7.8. Even buffer prepared from ripened coconut can sustain the motility of spermatozoa which goes to say that the nature of solution used to dilute the sperm appears to make no difference. Initial low levels of motility is enhanced by the addition of bovine serum albumin (BSA). In *Ephestia* (Norris 1932), *Bombyx mori* (Omura 1938) and *Galleria* (Khalifa 1950), the secretion is said to form the wall of the spermatophore while in *Cecropia* and *Pernyi*, these regions show no activity, region 2 (equivalent to EJ2) alone brings about the activity. Besides the common duct, no other part of the adult or developing moth contained the activator. As regards to the location of activity only EJ1 of *S. litura* and EJ1 and EJ2 of *H. armigera* regions of the common paired duct secretions exhibit sperm activity.

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Figures 1-19. *H. armigera*. 1. Distal part of the ejaculatory duct (EJ1 or simplex) in cross section exhibiting the muscle layer outer longitudinal (LM) and inner circular muscle (CM) layers, trachea (Tr), basement membrane (BM) and part of basal epithelium (EP) containing rough endoplasmic reticulum (rER). 2. Apical region in EJ1 showing numerous mitochondria (M). 3. Secretory epithelium in cross section of EJ1 exhibit elongated nuclei (N), highly active Golgi bodies (GB) and distended rough endoplasmic reticulum (rER). 4. EJ1 in cross section exhibiting apocrine secretory cell at the free cell surface, cell foldings (CF) extend deep into the lumen in the form of tongue like structures and apical membrane is thrown into extensive tight folds. 5. EJ1 in cross section exhibiting apocrine secretory cell at the free cell surface with intact epithelium (IEP) degenerating nucleus (dN) lumen (L). 6. Cross section in EJ1 showing the longitudinal muscle (LM) layer, circular muscle (CM), thick and prominent basement membrane. Note the nerve endings (NE) and trachea penetrate deep into the muscle layer nucleolus (NU) vacuole (V). 7. EJ1 merocrine secretory cell in cross section showing group of secretory vesicles (SV) and secretory granules (Sg). Note the granules are well defined, large, oval shaped and membrane bound containing electron dense materials (Edm) and matrix (MX). 8. Apical region in EJ1 showing lysed vesicles (Ly). 9. Cross section of EJ1 secretory epithelium exhibit rough endoplasmic reticulum actively involved in synthesizing materials. 10. EJ1 in cross section exhibiting apocrine secretory cell at the free cell surface accumulated materials (AM) and degenerating epithelium (EP). 11. Apical region in EJ1 showing apocrine secretory materials released into the lumen. 12. Cross section in EJ1 secretion granule formation (SF) point (SgP) rough endoplasmic reticulum (rER). 13. Cross section in EJ1 exhibiting apical region with numerous cell foldings (CF) and merocrine secretory materials are released. Note the pocketing (PC) secretions and the well pronounced microvilli (MV) with visible microtubules (Mt). 14. Cross section in EJ1 showing accumulation of granules light vesicles (LV). 15. Secretory epithelium in cross section revealing degenerating epithelium (dEP), degenerating rER and degenerating nucleus (dN). Note the granular secretory granules (GSg). 16. EJ1 revealing the topography of external muscle layers (ML) epithelium (EP). 17. EJ1 revealing formation of secretion (FS). (L, Lumen). 18. Topography of the lumen of EJ1 with its secretory granules (Sg). Note the hexagonal secretory granules accumulated in the lumen. 19. EJ1 revealing free cell surface (FCS) and epithelium (EP) are distinct.

Figures 20–34. *S. litura*. **20.** Cross section of EJ1 showing the muscle layers viz. outer longitudinal (LM) and inner circular (CM) layers. Note the thin, partially visible basement membrane (BM). Also note the secretory epithelium (SEP) with abundant rough endoplasmic reticulum (rER), occurring of apocrine secretion (AS) (* indicate the presence of special secretory granules). **21.** Cross section of EJ1 showing Golgi body (GB) and nucleus (N). **22.** EJ1 apical region in cross section exhibiting abundant rough endoplasmic reticulum. Note the merocrine secretion (MS) occurring and the accumulation of secretion(s) at the free cell surface (FCS) and released into lumen. **23.** Cross section showing highly secretory epithelium with rER and electron light vesicles (ELS) and concentric arrangement of rER and the distinct cell boundary. **24.** EJ1 exhibiting scattered mitochondria (M) and large nucleus (N) with distinct nuclear membrane (NM) and Golgi bodies (GB). **25.** Light micrographs from thick section exhibiting thick muscle layers (ML), epithelium (EP) and empty lumen (L), tall columnar cells with centrally located nucleus (N). **26.** Light micrographs from thick section lumen contains already some secretion. **27.** EJ1 showing large oval shaped nucleus (N) rER and apocrine secretion (AS). **28.** EJ1 showing numerous Golgi bodies (GB). **29.** EJ1, showing special secretory granules (Sg) of different sizes which are compartmentalised and located in the midst of highly secretory epithelium (SEP) rough endoplasmic reticulum (rER). **30.** EJ1 in cross section exhibiting apical region showing merocrine secretion (MS) at the free cell surface (FCS) with full of secretory material (SM) released from the accumulated secretory vesicles (SV). (L, Lumen). EJ1 in cross section exhibiting 6 different types of SV-1 (**31**), SV-2 (**32**), SV-3 (**33**), SV-4, SV-5, SV-6 (**34**). Note the membrane bound as well as membraneless and the differential content in them.

Fine structure of the regional differentiation of the ductus ejaculatorius simplex (EJ1, EJ2, EJ3) along with the role of their secretions in sperm activation and motility in two Noctuid species, *Heliothis armigera* (Hübner) and *Spodoptera litura* (Fabricius) (Lepidoptera). II. Fine structure and function of simplex 2 (EJ2)

G AMALDOSS

Biology Department, Fu Jen University, Hsinchuang 24205, Taipei, Taiwan, Republic of China

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Abstract. The mid region (EJ2) of common ejaculatory duct is characterised by secreting intensity varying white viscous secretion together with a small segment of the milky white viscous secretion. The milky white segment possesses as the optimum amount of activator, high molecular weight protein between 30,000 and 60,000 capable of disaggregating eupyrene sperm bundles besides possessing the activating substance.

Keywords. Viscous secretion; sperm disaggregation; activator.

1. Introduction

The spermatophore is not a mere repository or transport vehicle for spermatozoa (Mann 1984) but a reactor for spermatozoan maturation (Katsuno 1977; Kasuga *et al* 1985). Maturation precedes with the time of acquisition of a nucleated apyrene spermatozoa and the breakdown of eupyrene bundles is due to the separation of individual eupyrene spermatozoa that fertilize the eggs and their partial acquisition of motility which is completed later in the spermatheca (Kasuga *et al* 1987). In this paper the fine structure and role of EJ2 secretion in sperm activation, motility and sperm disaggregation *in vitro* have been described.

2. Materials and methods

The animals were reared, dissected, embedded, sectioned and stained as described by Amaldoss (1987). Preparation of sperm, activator and assay of sperm activation are carried out as described previously by Shepherd (1972).

2.1 Purification and characterisation of sperm activating substances using gel filtration technique employing Sephadex (G-50)

2.1a Preparation of buffer (pH 6.8): Ammonium bicarbonate, 250 ml of 0.03 M was added to 250 ml of 0.03 M acetic acid; to this mixture 500 ml of double distilled water was added, and thus the buffer attained 0.03 M for each ingredient.

2.1b Preparation of column: Sephadex G-50 (fine beads) from Pharmacia Fine Chemicals, Inc., Uppsala, Sweden was hydrated by heating the beads in the buffer

for 1 h in a boiling water bath. The gel was washed until the absorption of the eluent at 280 nm was <0.005 .

2.1c Preparation of the sample: The insects were dissected open in Weever's (1966) lepidopteran saline and the common ducts of 5 insects of each species were pooled and homogenized in 0.03 M ammonium bicarbonate/acetate buffer, centrifuged and the supernatant was used for gel filtration.

2.1d Marker proteins: One of five $\mu\text{g/ml}$ of solutions of various markers of known molecular weights (M_r) were applied to the column. These markers were bovine serum albumin (M_r , 68,000), cytochrome c (M_r , 12,100), glucagon (M_r , 3485), bacitracin (M_r , 1450) and leucine (M_r , 131).

2.1e Procedure: The common duct extract was applied to the equilibrated column, and the eluents were collected at a flow rate of 0.2 ml per min. The eluents were read at 280 nm. The fractions which showed same peaks at 280 nm were pooled and lyophilised and tested for sperm activation.

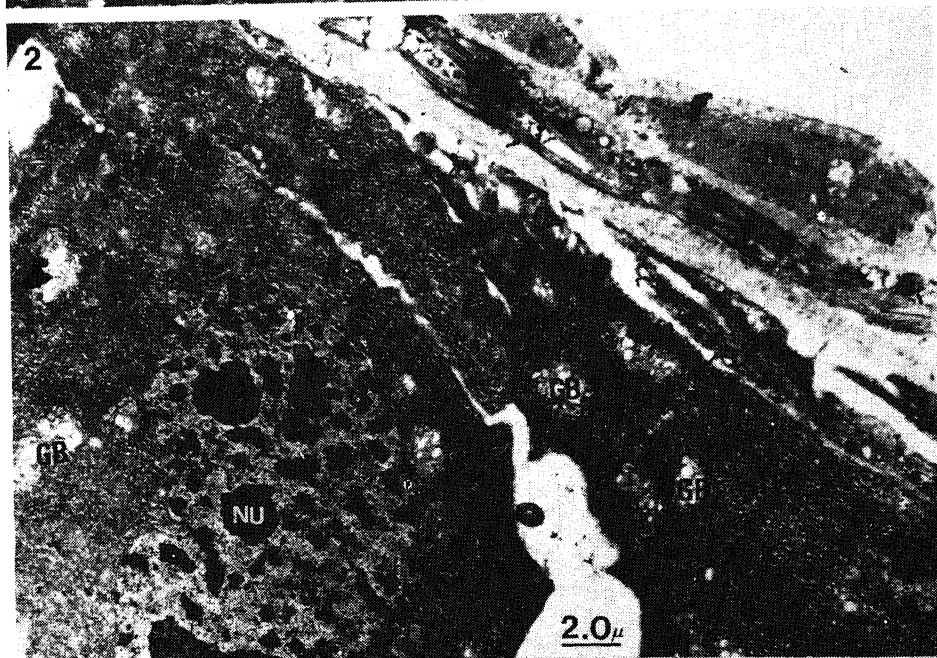
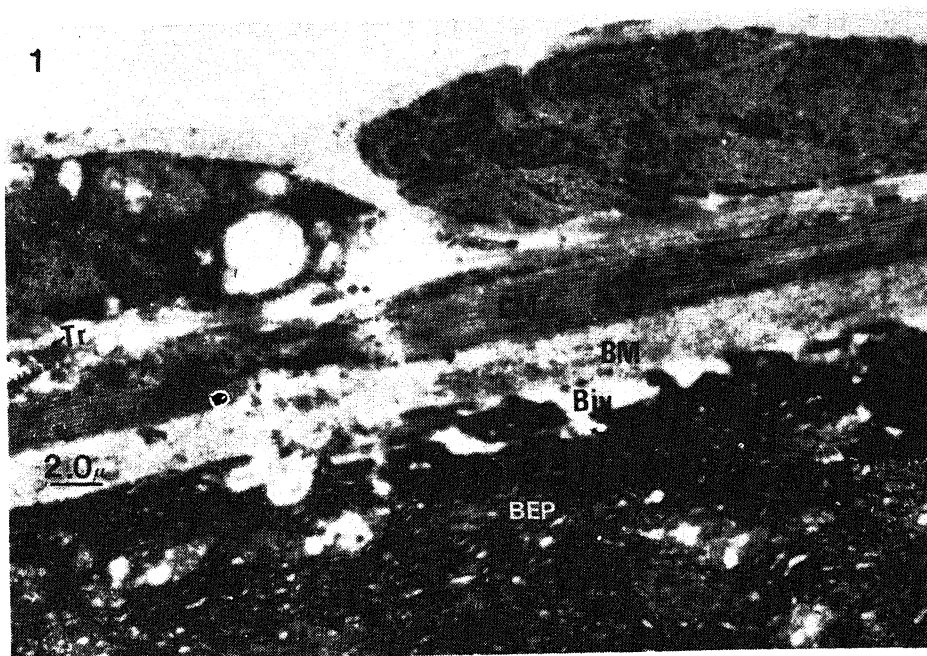
After eluting the sample, the Sephadex G-50 column was washed with 0.3 M ammonium bicarbonate/acetate buffer until the eluent read 0.005 absorbance at 280 nm. Then the marker substances were eluted at a flow rate of 0.2 ml/min. The elution volume of leucine was determined by ninhydrin test.

3. Results

EJ2 is in the second or mid region of the non cuticular simplex containing varying intensity from white, bright white and milky white secretions as it descends from EJ1 to EJ3 region of the common ejaculatory duct. The length of the milky white segment is identical in both the species. Interestingly the milky white segment shows copious sperm activity in both the species. The length of EJ2 in *Heliothis armigera* measures 2.6 cm while the same in *Spodoptera litura* measures 2.4 cm. The secretory epithelium consists of low columnar cells in the EJ2 of *H. armigera* while the same consists of tall columnar cells in the EJ2 of *S. litura* (figure 17). The secretory activity appears to be quite different from that of EJ1. The secretions appear to accumulate in vacuoles at the free cell surface where the vacuoles rupture and the contents are emptied into the lumen (figures 27, 29).

Scanning electron micrographs (SEM) of the cross sectioned EJ2 of the simplex reveal a thick outer epithelial layer which is smooth and appears to show secretory cell surface of apocrine nature (figure 8). Though there is a single secretory cell type, there appears topographic variations as if there are two types of secretory cells. The basal region appears much larger than the apical region. The apical region exhibits dark, dense secretory granules and they are seemingly released by exocytosis. The lumen consists of homogenous moderately electron light secretory materials besides dark, dense secretory granules (figures 9, 10). The cell appears highly secretory phased. Dense cored secretory materials are at the centre around which there is an electron lucent material enveloping the materials. Some granules appear to be membrane bound while others are not so (figure 4). Under the light microscope the section appears milky white and thus supplements the information gathered and SEM. At the ultrastructural level, the epithelium of both the EJ2 species *H. armigera* and *S. litura* consists of a single cell type (figures 1, 2, 12-14).

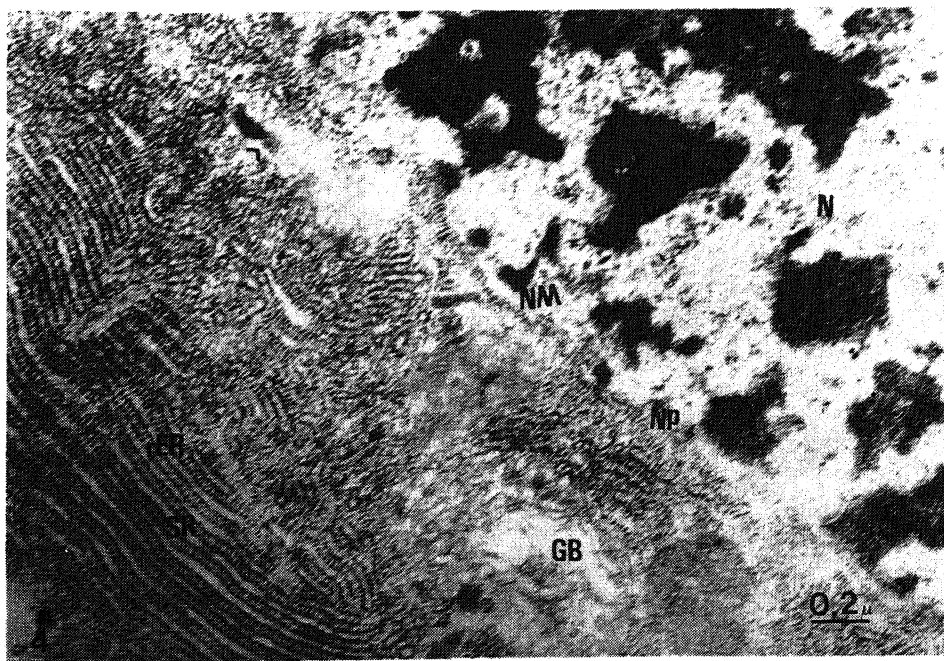
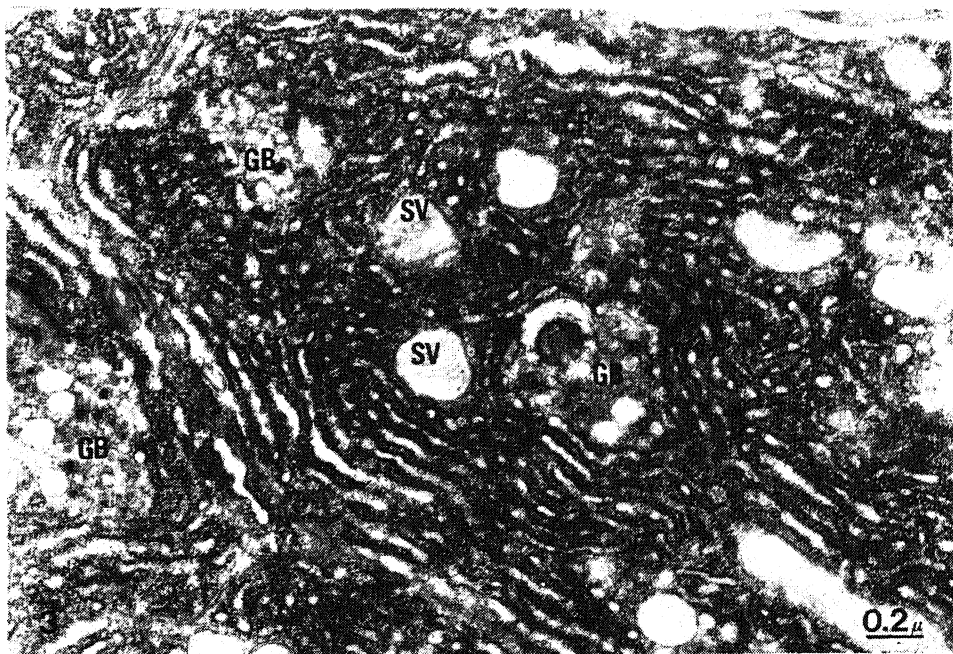
Apocrine appears to be the only mode of secretion in *H. armigera* (figure 7) while the epithelium of EJ2 in *S. litura* appears to have two secretory cell types. A larger granule secreting basal epithelium with characteristic multivesicular bodies exhibit apocrine mode of secretion while apical region appears to function holocrine type



Figures 1 and 2. For caption, see p. 37.

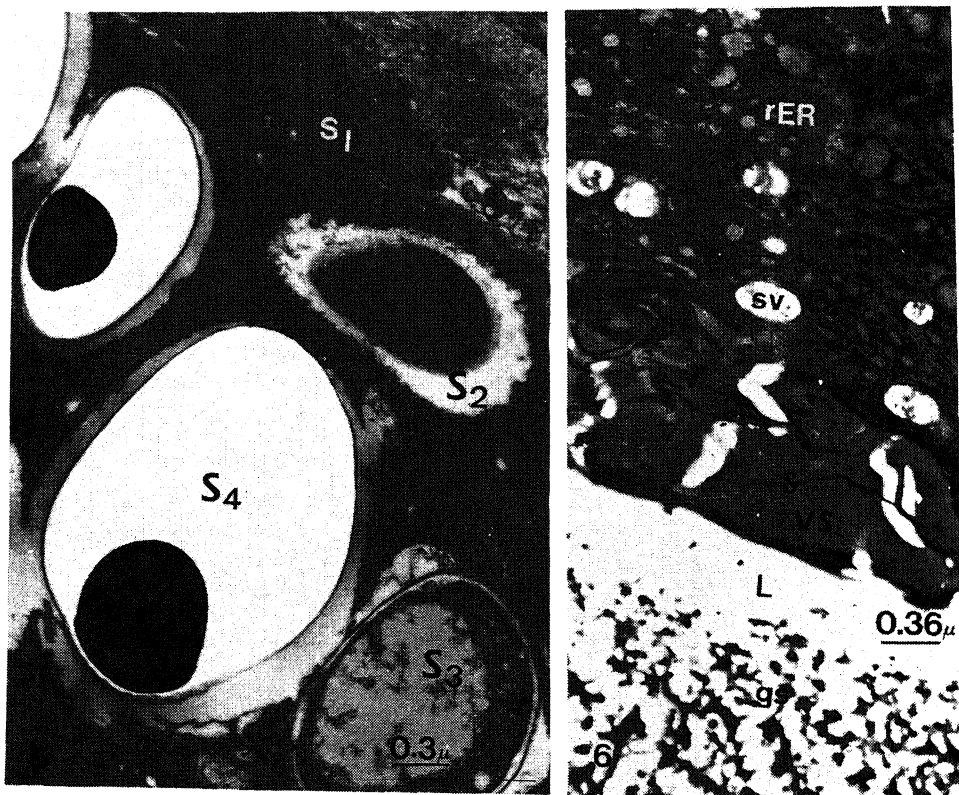
of degeneration wherein all the cell contents are lysed and expelled into the lumen to form the part of the ejaculate (figures 20-23).

The secretory epithelium of EJ2 both in *H. armigera* and *S. litura* are like that of EJ1 in having two muscle layers surrounding the basement membrane. An outer

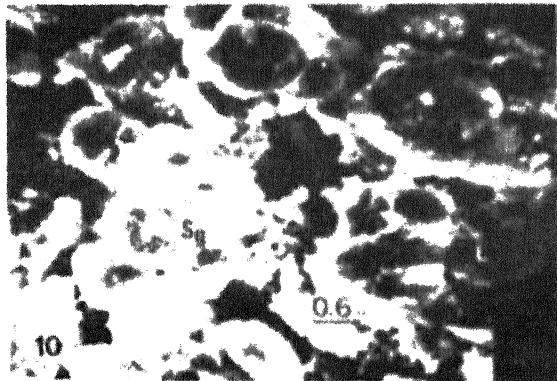
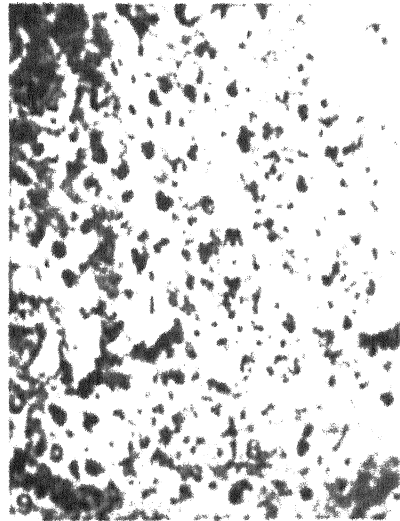
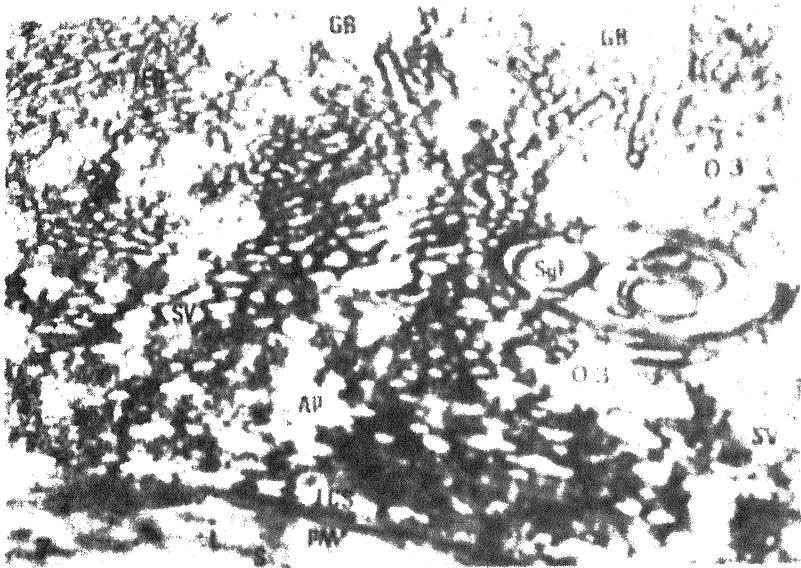


Figures 3 and 4. For caption, see p. 37.

thick longitudinal muscle layer and an inner thin circular muscle layer are the two layers that surround the basement membrane and epithelium. The basement membrane of EJ2 in *H. armigera* appears to be much thicker than the same of EJ2 in *S. litura* (figures 1, 2, 12, 13). The epithelium of EJ2 region of the simplex in the case of *H. armigera* consists of only a single cell type, viz. apocrine. The secretory epithelium consists of smooth endoplasmic reticulum and rough endoplasmic reticulum. The cytoplasmic matrix is darker than that of EJ2 epithelium of *S. litura* (figures 2–4). The nuclei which are oval, are located basally and each of them contains 3 or 4 nucleoli. Dark dense nuclear materials are evenly distributed. The nuclear membrane does not appear conspicuously (figures 2, 4). Golgi bodies and mitochondria are evenly distributed. The rough endoplasmic reticulum is distended and vesiculate. The apical region is filled with abundant electron light secretory vesicles (figures 3, 4). The apical region has a smooth surface at its free cell surface (figures 6, 7). There is no sign of microvilli present. The secretory materials are released at the free cell surface by breaking the cell membrane with cytoplasmic loss. The lumen contains the granular secretory materials (figure 7). The epithelium is characterised by the presence of secretory vesicles peculiar to the cells (figure 5). Four types of secretory granules designated S1, S2, S3 and S4 are recognised. The first type (S1) has tapering ends and its contents are moderately electron dense. The outer surface of this secretory granule



Figures 5 and 6. For caption, see p. 37.



Figures 7-10. For caption, see p. 27

consists of thick, dark and electron dense materials. The second type (S2) of secretory vesicle contains dense cored materials in the centre around which there is an electron lucent space enveloping and forming a boundary. The third type (S3) is the secretory vesicle which is membrane bound with an electron lucent space which again encloses moderately electron dense material. The inner most part of this secretory granule contains electron lucent materials with granular materials scattered. The fourth type (S4) of the secretory granule is also membrane bound. Beneath this membrane is an electron lucent space which, in turn, encloses a dark, spherical electron dense material of a secretory nature. The apocrine secretion at the free cell surface is expelled into the lumen of EJ2 with cytoplasm (figure 7). The muscle layers of EJ2 in both the species are deeply penetrated by the nerve endings and tracheae which however do not penetrate the epithelium. The nuclei of the muscle layers are elongated. Both the muscle layers contain dense cored vesicles. EJ2 of both species have basal invaginations which is not a characteristic feature of EJ1 (figures 1, 2, 12, 13).

In the apocrine secretory cell type of *S. litura*, the epithelium consists of abundant rough endoplasmic reticulum (figures 15, 16, 18, 19, 22, 23, 25). The rough endoplasmic reticulum is further arranged in whorls surrounding secretory vesicles and other secretory materials. There are Golgi complexes as well (figures 15, 16, 18, 19). In other areas of the cells where the endoplasmic reticulum and secretory vesicles are scanty, the Golgi bodies and the mitochondria are sparse and randomly distributed. Numerous electron dense and light secretory vesicles are also present. The rough endoplasmic reticulum is lamellate but sometimes distended. The unique feature of this region is the formation of secretory granules. The nuclei are rounded and the nuclear membrane clearly demarcated. The rough endoplasmic reticulum may also become distended. A variety of secretory vesicles occur, and they consist of homogeneous dense materials which are separated from the surrounding membrane by means of an electron lucent space (figure 11). Secretory materials appear to be released by exocytosis at the bases of free cell surface. There is no clear evidence of any microvilli at this region of the cell. The lumen contains electron dense-light vesicles and multi-vesiculate bodies (figures 26, 28).

In the holocrine cell type the epithelium is distinct with cell boundaries (figure 23). The plasma membrane is clearly demarcated and regularly arranged. The nucleus is more or less oval in shape and the double layered nuclear membrane is partially visible. The nuclear materials appear homogeneous and there are 4 or 5 nucleoli in each nucleus. The cells have many electron lucent vesicles, both rough and smooth endoplasmic reticulum, Golgi complexes with associated dense vesicles, a few mitochondria and some irregularly shaped materials. The characteristic feature of this region is the presence of multivesiculate granules, and crystalline structures occurring in the electron lucent vesicles. The electron lucent vesicles are of different sizes and shape and contain crystalline structures. At the outer region of this unique secretory vesicle is a boundary of electron light region, followed by the cytoplasmic inclusions of the cell. Another inclusion of this secretory granule is the rod-like material (figure 22). The whole secretory epithelium undergoes drastic changes during reproductive phase. Degenerating endoplasmic reticulum of both smooth and rough types and other cell organelles are readily changed and converted for transport into the lumen. The degenerating epithelia form different layers of materials with intensity of low and high orders (figure 25). The

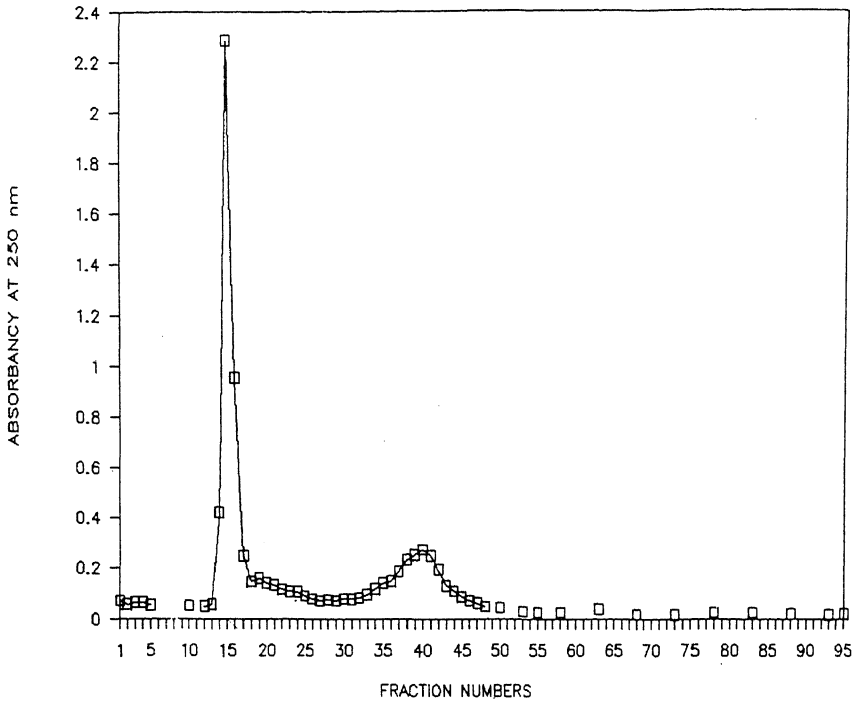
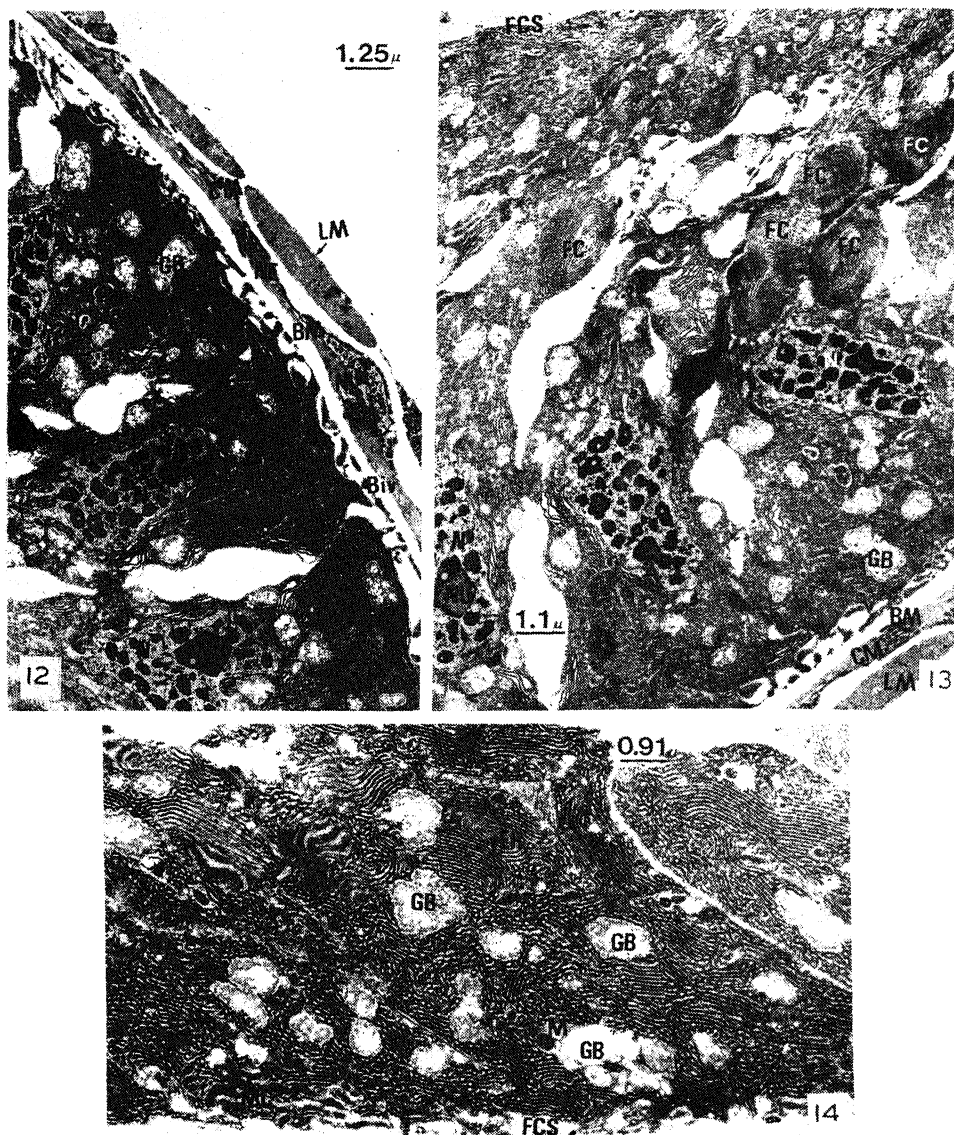


Figure 11. Gel filtration of EJ2 extract of *H. armigera* on Sephadex G-50.

region close to the apex and lumen appears moderately electron dense and contains mitochondria, free ribosomes, pieces of endoplasmic reticulum and many types of secretory vesicles. Prominent among these secretory vesicles are the electron light and electron dense types. There are also dark, dense cored materials and microtubules. The free cell surface of the apical region is clear and smooth and presents an undulate outline along its entire length (figure 24). The next region is highly electron dense, containing materials mostly like those of the previous region. It has a clear boundary. The distal region is the electron luscent part which has less materials. There are large empty spaces in this region. Secretory vesicles, mitochondria and broken pieces of rough endoplasmic reticulum are scattered here and there, and it appears that the holocrine secretion takes place in the sequential space. The materials get gradually but steadily pushed towards the apical region only to be expelled into the lumen subsequently.

3.1 *In vitro* purification and characterization of the sperm activator from Noctuid moth

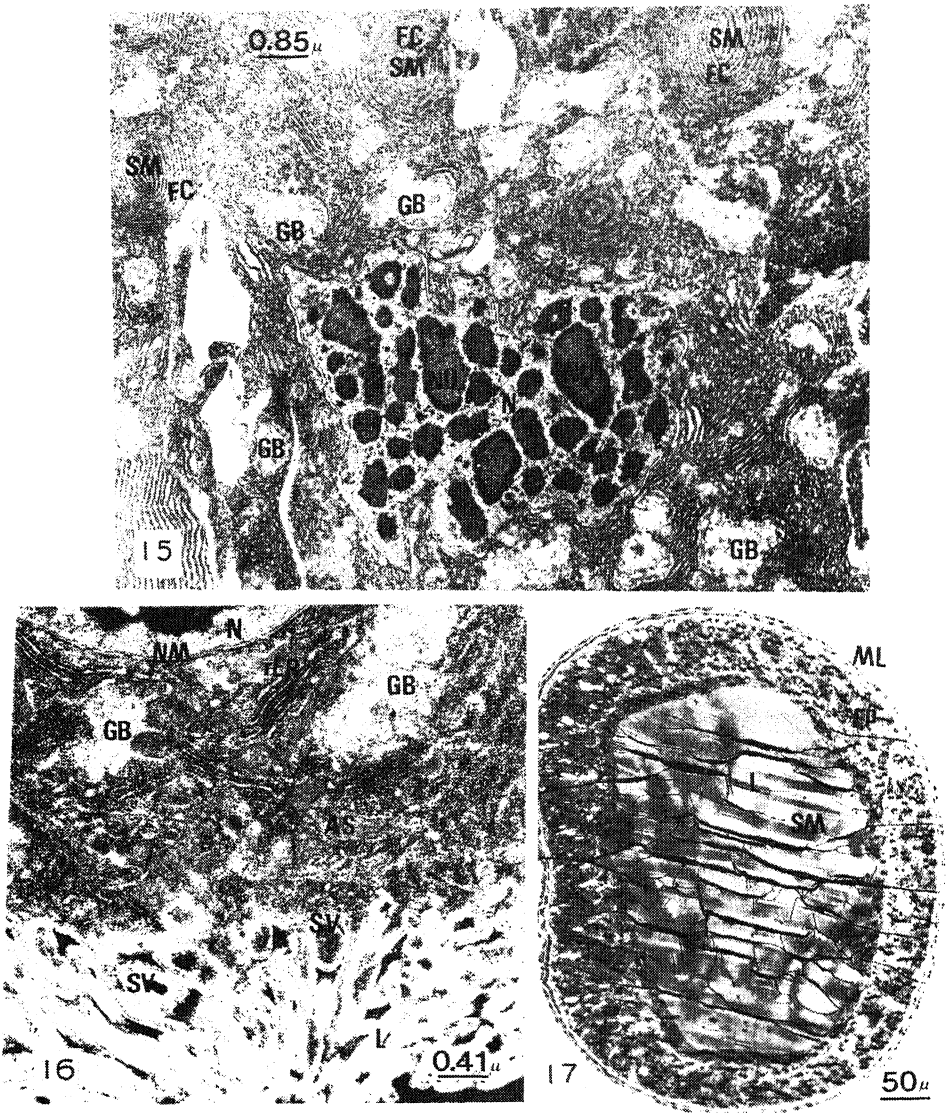
It has been observed that as long as the sperm are in the seminal vesicles, they are practically immotile, but become highly motile on entering the female reproductive tract upon copulation. Thus, during copulation secretions from the accessory gland should play a major role in the initiation of sperm motility. In order to understand which region of the male reproductive system secretes the activator



Figures 12-14. For caption, see p. 38.

of the sperm, the homogenised contents from various regions of the male reproductive tract, viz. duplex, accessory reproductive gland, vas deferens and the simplex were mixed with the sperm dissected from the seminal vesicle and nature of sperm activation was observed. Prior to this, dilution of the sperm with buffer solution is essential to assay the effect of the secretions. Various buffers at different pH in different ionic strengths were used as dilutants.

The results obtained on the effect of various molar concentrations of HEPES-KOH buffer on sperm activation of *S. litura* indicate that there is no activity of sperm in 0.03 and 0.05 M HEPES buffer. Activation is noticed at 0.16 M buffer. Sperm

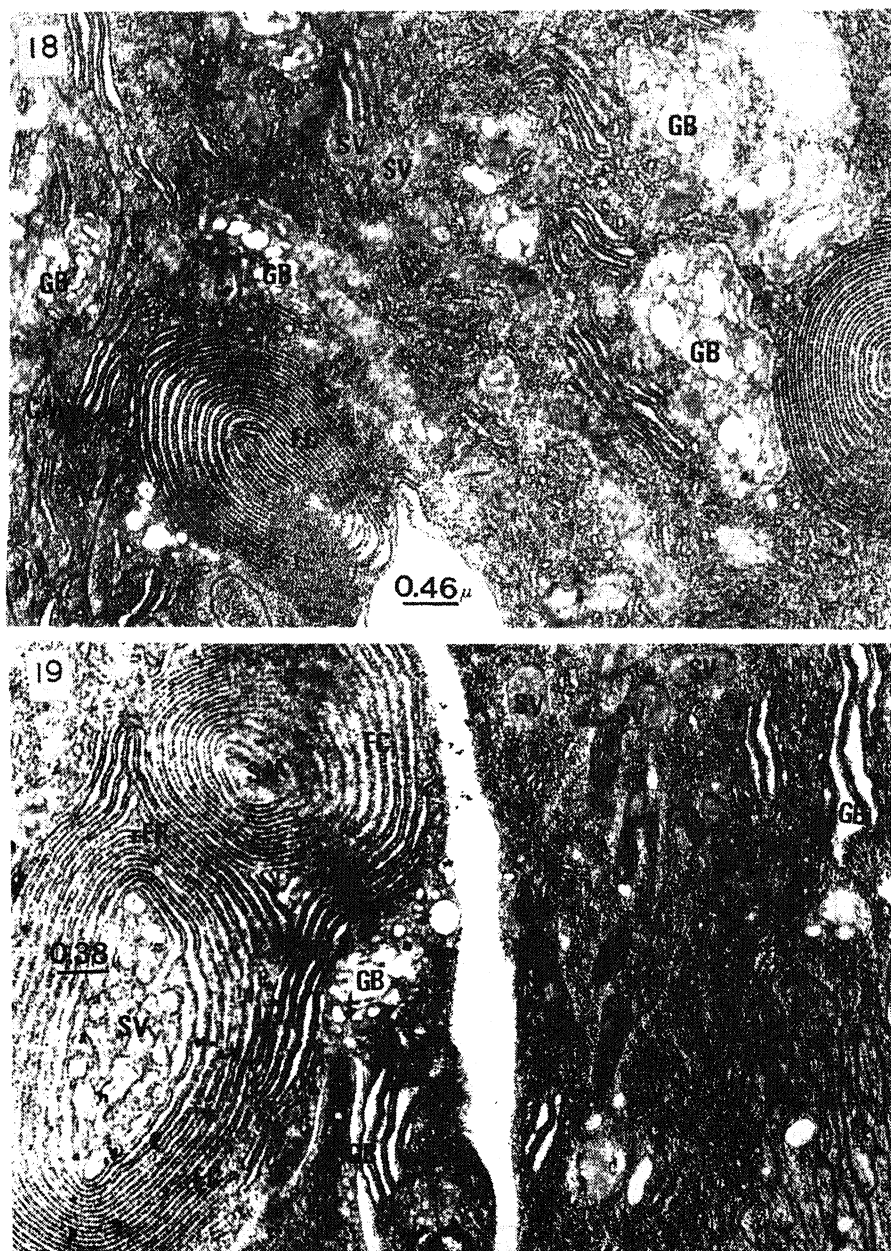


Figures 15-17. For caption, see p. 38.

activation is reduced when the buffer concentration is increased above 0.16 M, while complete inhibition of activity is observed at 0.5 M concentration. The assay of sperm activation in different pH in HEPES buffer further reveals that the activity is maximal at pH 7. In view of this, HEPES buffer (0.16 M) at pH 7 was used as dilutant. The same buffer at pH 7 at 0.16 M was found to be suitable for the activation of the sperm of *H. armigera* as well.

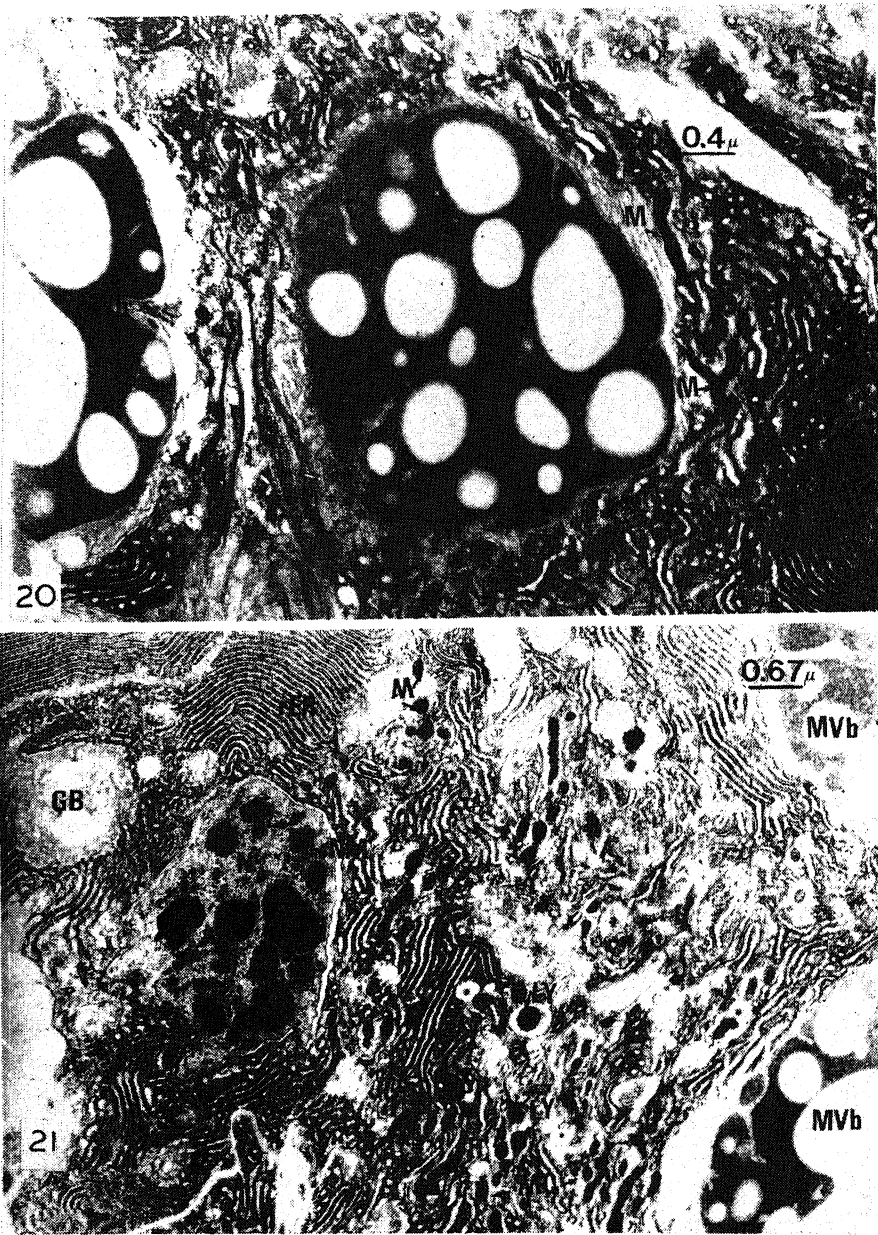
3.2 Distribution of activator in male accessory gland secretions

The results obtained on the effect of the secretions of the seminal vesicle and EJ1 to



Figures 18 and 19. For caption, see p. 38.

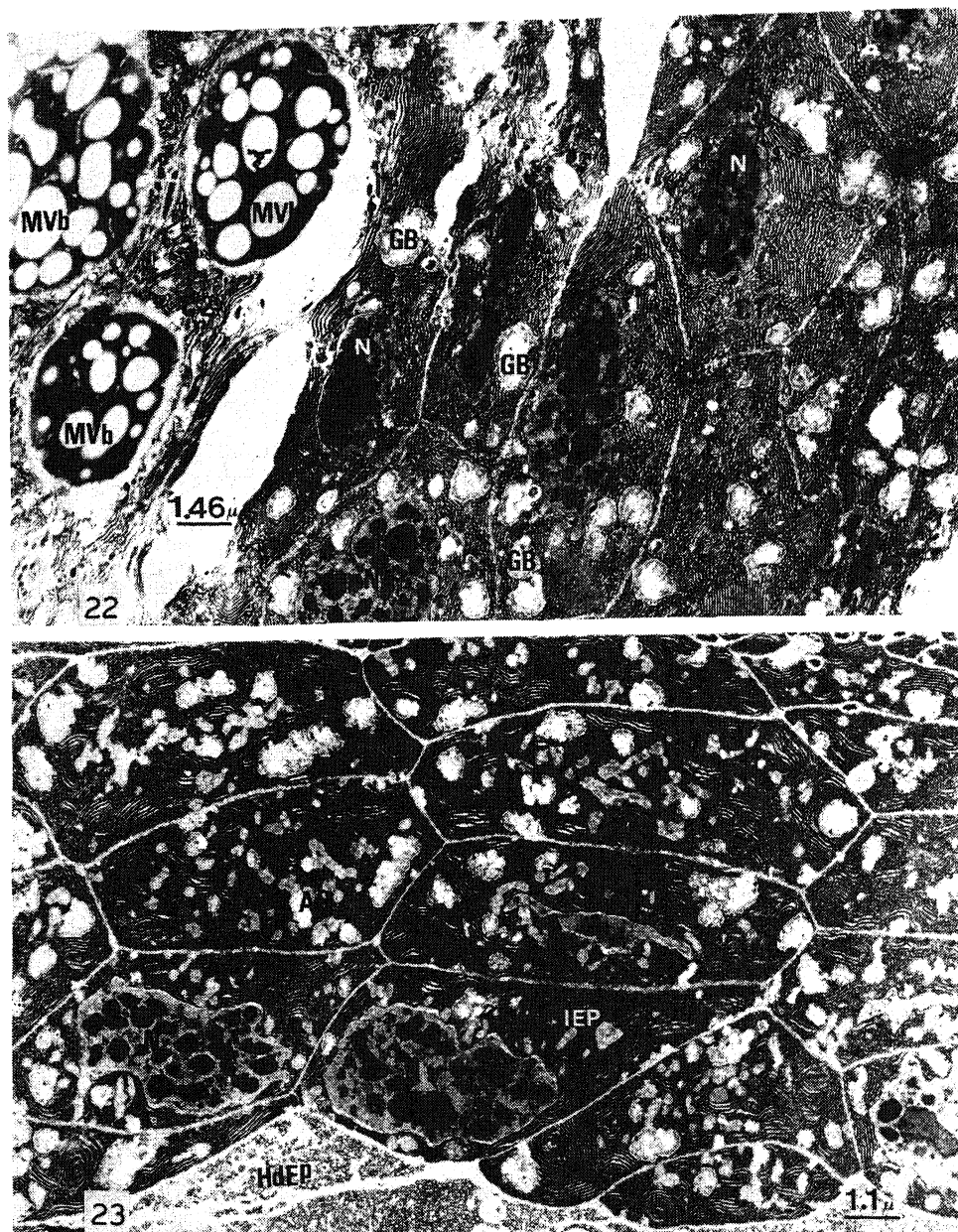
EJ2 are presented in table 1. It is found that in the presence of HEPES buffer, the seminal vesicle has no effect on the activation of sperm. However, extracts from different regions of the common duct or simplex activate the sperm to varying degrees while EJ2 extract activates the sperm maximally.



Figures 20 and 21. For caption, see p. 38.

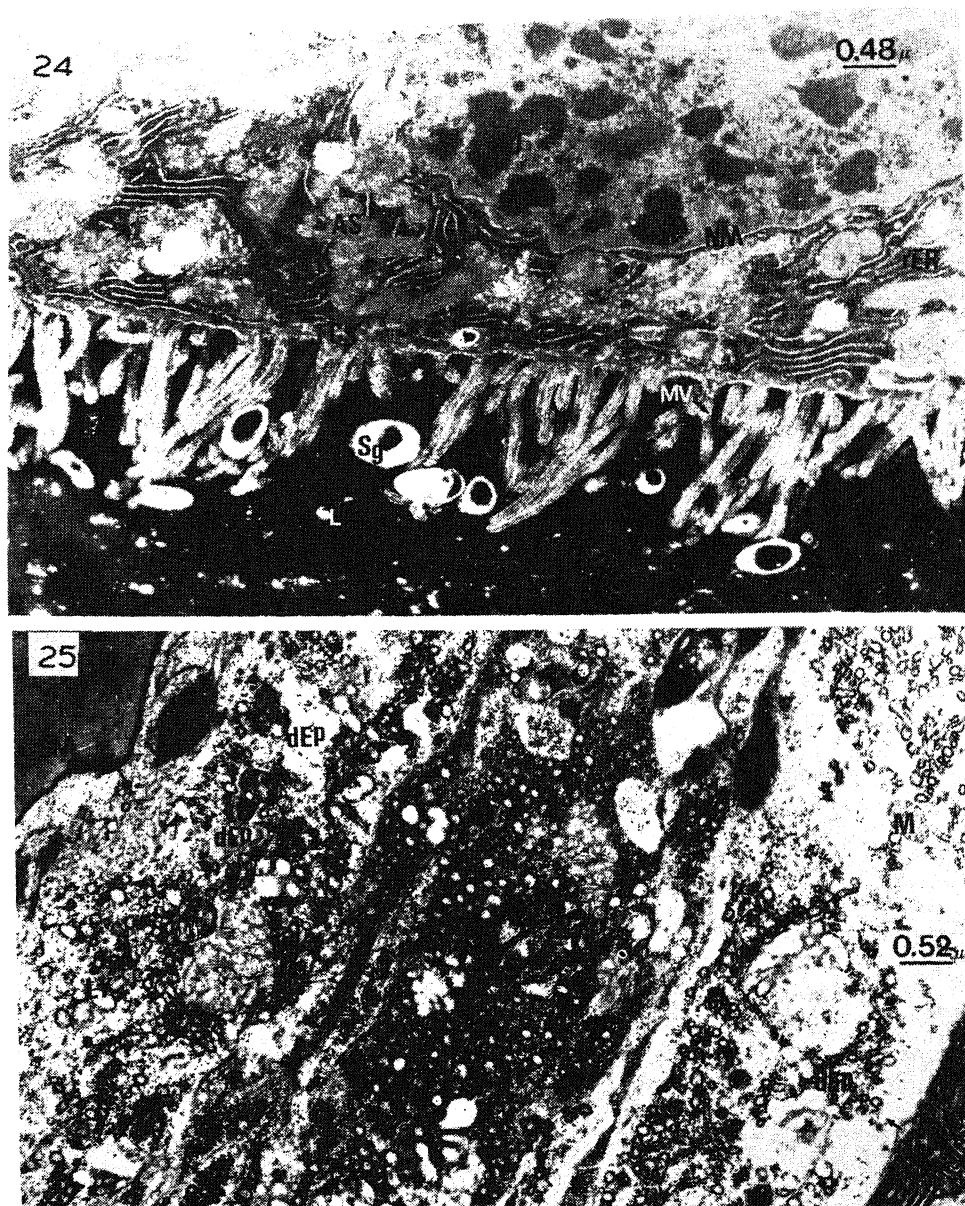
3.3 Chemical properties of the sperm activator

To understand the nature of the chemical composition of the secretion that activates the sperm in both the insects under study, the secretions were subjected to UV spectrum analysis. The secretion of the common ejaculatory duct or simplex was diluted with HEPES-KOH buffer at pH 7 and centrifuged at 1000 *g* for 1 h.



Figures 22 and 23. For caption, see p. 38.

The supernatant was measured with a spectrophotometer. The results obtained on the absorption spectrum of the secretion of *S. litura* and *H. armigera* show the maximum absorption of the secretion at 270–280 nm suggests the presence of tyrosyl groups of the protein of the secretion that are responsible for sperm activation and motility.

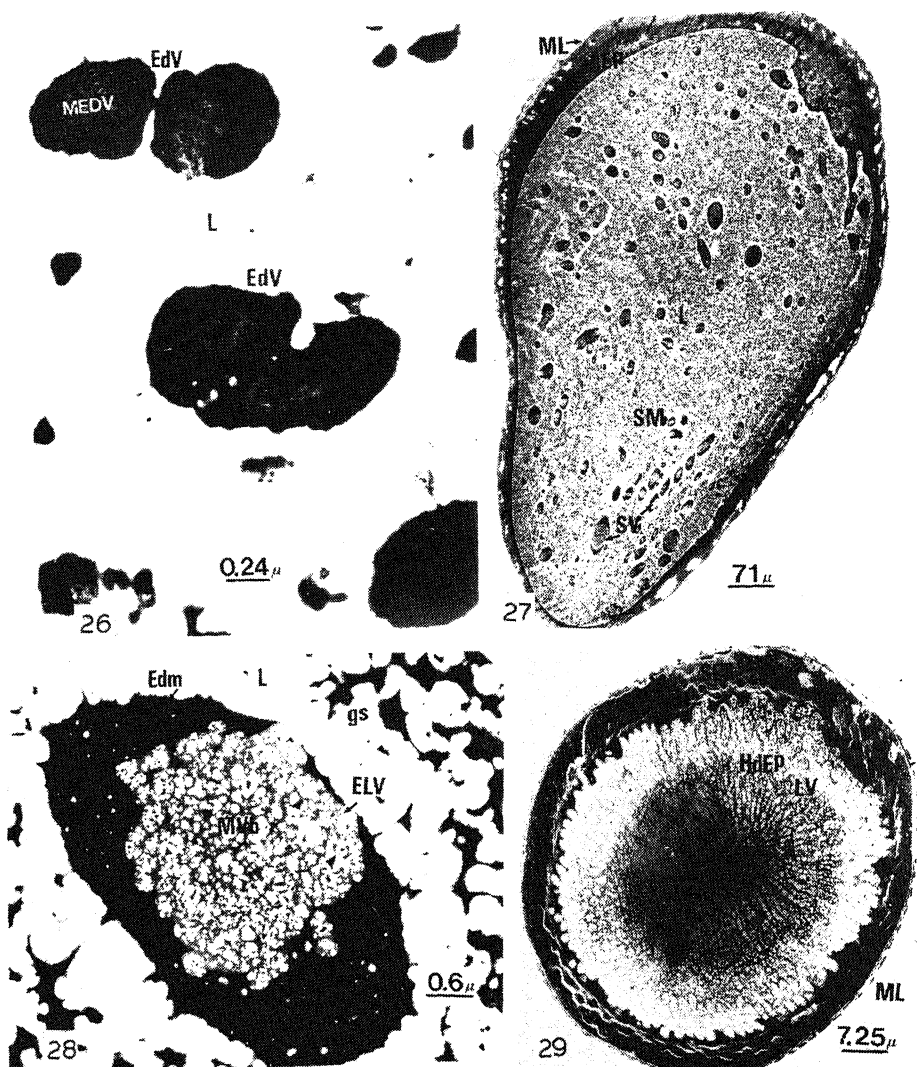


Figures 24 and 25. For caption, see p. 38.

3.4 Susceptibility of activators to proteolytic and hydrolytic enzymes

The results obtained on the susceptibility of the activator to proteolytic and hydrolytic enzymes are presented in table 2. It is observed that the sperm activation capacity of the secretions from the simplex gland of *S. litura* and *H. armigera* are inhibited by proteolytic enzymes such as trypsin, subtilisin and pepsin.

There is no inhibition of the activator when the secretions were incubated in heat-denatured proteolytic enzymes. Incubation of the activator with non-proteolytic



Figures 26-29. For caption, see p. 38.

enzymes such as hyaluronidase and ribonuclease does not alter the property of the activator. The susceptibility of the activator to proteases and non-susceptibility of the same to non-protease enzymes further suggest that the activator is a protein or peptide. The spectral property adduces further evidence to this conclusion.

To understand whether the activator is a high M_r protein or a low M_r peptide, gel filtration study was conducted. The results as presented in figures 11 and 30 reveal that only two fractions were separated in Sephadex G-50 in both the insect species studied. The first fraction was separated in the void volume (fraction No. 12-20) and this suggests that M_r higher than 30,000 are excluded at this region. The second small peak was observed between the fraction No. 35-45. In this region, vitamin B12 was excluded, suggesting the polypeptide responsible for activation having a M_r lower than 5000 (dalton). In *S. litura*, though the first fraction was separated in

Table 1. Sperm activation in various media.

Sperm	Saline	Seminal vesicle extract	EJ1	EJ2	EJ3	HEPES-KOH	Result
x	x						—
x		x					—
x			x				—
x				x			—
x					x		—
x						x	—
x	x		x				+++°
x	x			x			++++
x	x				x		+++
x						+	—
x		x	x				+++
x		x		x			++++
x		x			x		+++
x		x				x	—
x			x			x	+++
x					x	x	++++
x					x	x	+++

—, No activity; +++, intense activity; +++, very intensive activity; +++°, intense activity only in *H. armigera* and not in *S. litura*.

Table 2. Susceptibility of sperm to proteolytic and hydrolytic enzymes.

Proteolytic enzyme	
Trypsin	+
Subtilisin	+
Pepsin	+
Non-proteolytic, hydrolytic enzymes	
Hyaluronidase	+++
Lysozyme	--
Ribonuclease	+++

+, Very less active; +++, very active; —, no activity.

void volume, the second peak was separated between the fraction No. 45–50, suggesting the M_r of the sperm activator being slightly less than that of *H. armigera*.

4. Discussion

The simplex of *S. litura* and *H. armigera*, as stated earlier, is divided into 3 segments, viz. EJ1, EJ2 and EJ3, on the basis of their functional morphology and their secretions responsible for sperm activation and sperm motility. The first two segments are non-cuticular while the third segment is cuticular in as much as it has a lining of intima. The 3 segments also differ in their relationships with regard to the secretory epithelium, basement membrane and muscle layer. EJ2 are not lined by an intima and their muscle layers are intimately associated with the epithelium

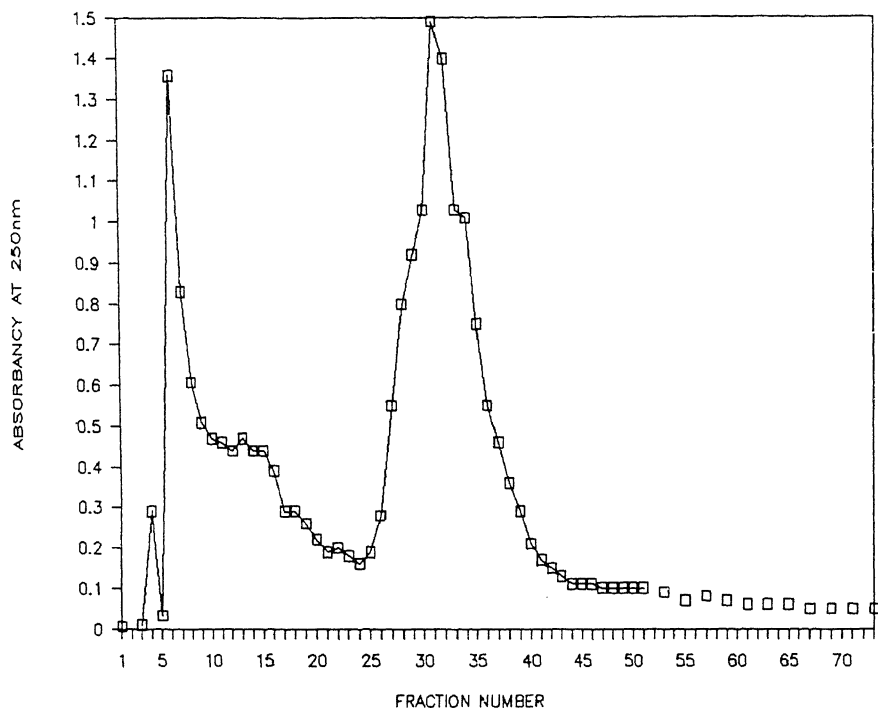


Figure 30. Gel filtration of EJ2 extract of *S. litura* on Sephadex G-50.

as shown by the 'fusion' of their basement membranes. The most notable feature in the arrangement of the muscle layers in the 3 segments of *S. litura* and *H. armigera* is the uniformity, in having a thin, scant inner circular muscle layer, and a thick, robust outer longitudinal muscle layer, whereas in other species of Lepidoptera studied, there are differences in the wall of the simplex in the matter of the presence or absence of the muscle layers, and the relative thickness of the layers, when muscle layers occur. For instance, Musgrave (1937) reported the absence of muscle layers in the simplex in *Ephestia kuhniella*. Brits (1978) did not find any muscle layers in *Phthorimaea operculella*, while Riemann and Thorson (1979) observed thin layers of muscles around atleast a portion of the simplex. In the arrangement of the muscle layers, *S. litura* and *H. armigera* approach that of *Calpodex ethlius* reported by Lai-Fook (1982) very closely, with the circular layer of muscle always inner in position and thin and scant, while the longitudinal layer of muscle always outer in position and thick and robust. Further, as in *C. ethlius*, the two species studied here show an abundance of nerve endings and tracheae entering the muscle layers of the simplex throughout its length.

In the EJ2 of *S. litura* both apocrine and holocrine types of secretion are noticed, while in *H. armigera* this region of the simplex shows only apocrine cell type. EJ2 exhibits special types of secretory granules only in *S. litura*, and they are concerned with spermatophore formation, sperm activation and sperm motility. EJ2 of both the species studied here is characterised by the presence of secretory vesicles peculiar to the cells.

4.1 Sperm motility and activity

The available evidence on vertebrates (Mann 1964; Salisbury 1962; Nelson 1967) and sea urchins (Rothschild 1956; Rothschild and Cleland 1952) indicates that the sperm are primarily activated during ejaculation. The reason for this is that the sperm are provided with metabolic substrates during ejaculation. The sperm removed from the storage organs (SV and duplex) *S. litura* and *H. armigera* could be activated neither by the substrates nor by exposure to oxygen as the sperm of saturniid (Shepherd 1972). Although other factors like change in ion concentration and pH are reported to have some impact on the activation of sperm in some insects, *S. litura* and *H. armigera* sperm have no impact from these factors. The present investigation clearly showed the presence of activator substance in the unpaired gland in both the species of Noctuidae. This observation is in agreement with that of Shepherd (1972) who found similar results in Lepidopteran and Orthopteran species. Purification and characterisation of the activator substances using gel filtration showed that the activator has two components with high and low M_r . *In vitro* studies of sperm activity using the two components of activator showed that the high M_r component seemed to disaggregate the sperm bundles while the low M_r component seemed to concern only about sperm activity and motility.

The two components of activating substance seem to be protein in nature. The component that disaggregates the sperm bundles is estimated to have the M_r ranging between 30,000 and 50,000 on Sephadex columns for *S. litura* and *H. armigera*. The low M_r component is estimated to have less than 50,000 dalton in respect of *S. litura* while the same appears to be further slightly lower in the case of *H. armigera*. Further chemical analysis of the two components of both species can reveal their structure and even the mechanism. The protein nature of the substance (say a polypeptide) can also be sequenced which again will reveal the structure.

In EJ2 of *S. litura* both apocrine and holocrine types of secretion are noticed, while in *H. armigera* this region of the simplex shows only apocrine type. EJ3 of both *S. litura* and *H. armigera* is secretory in nature through microvillate surfaces, and by exo and endocytosis the materials are expelled into the lumen, and the secretion is only of the apocrine type. EJ2 exhibits special types of secretory granules only in *S. litura*, and they are concerned with spermatophore formation, sperm activation and sperm motility. EJ2 of both the species studied here is characterised by the presence of secretory vesicles peculiar to the cells.

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Figures 1–10. *H. armigera*. **1.** EJ2 in cross section exhibiting outer longitudinal muscle (LM) layer, inner circular muscle (CM) layer and a thick prominent basement membrane (BM). Note the trachea (Tr) and nerve endings into the muscle layer. Also note characteristic basal invaginations (Biv) and dense basal epithelium (BEp). **2.** EJ2 in cross section exhibiting oval nucleus, 3 or 4 nucleoli (Nu), Golgi body (GB) and clearly demarked cell boundaries. (NU, Nucleolus; SEP, smooth endoplasmic reticulum). **3.** EJ2 in cross section showing actively synthesizing secretory epithelium. Note numerous Golgi body (GB), dilated tubular rough endoplasmic reticulum and secretion accumulating vacuoles (SV). **4.** EJ2 secretory epithelium exhibiting ER with richly studded ribosomes. Note oval shaped nuclei with dense chromatin materials. Note also double nuclear membrane (NM) with nuclear pores (NP). **5.** EJ2 in cross section shows distinct and characteristic secretory vesicles S1, S2, S3 and S4 with differential material content and thereby signifying their functional roles. **6.** EJ2 apical region in cross section showing the rich apical region supplied with packaging and transporting secretory material and secretory vesicles (SV). Note the rough endoplasmic reticulum (rER) are vesicle shaped. Note also the lumen (L) with granular secretory (gs) materials and free cell surface containing viscous secretion (VS). **7.** EJ2 in cross section exhibiting the apical region already noted in figure 6, the packaging and transporting of viscous secretion and expelling them into the lumen (L). Note the Golgi body (GB) involved in synthesis, the vesiculate rER and numerous secretory vesicles (SV). Also note the accumulation of secretion containing vesicles (SV) at the free cell surface (FCS) and clearly demarked plasma membrane (PM). It is noteworthy to see secretory granule formation (SgF). **8.** EJ2 exhibiting topography of muscle layer (ML), secretory epithelium (SEP), apical region (AR) and secretion filled lumen (L). **9.** EJ2 lumen showing characteristic granular and viscous secretory material (SM). **10.** EJ2 lumen exhibiting granular viscous secretion in the form of secretory granules.

Figures 12-29. *S. litura*. 12. EJ2 in cross section exhibiting longitudinal muscle layer (LM), inner circular muscle layer (CM) and thin prominent basement membrane. Note the muscle nucleus (MN) and nerve endings (NE). Also note the basally located oval shaped nuclei with fewer nucleoli (Nu). 13. EJ2 in cross section exhibiting rich rER as well as finger like configuration (FC) or concentric whorl rER. Note the characteristic basal invagination (Biv). (GB, Golgi body). 14. EJ2 in cross section showing abundant rER, Golgi body (GB) and mitochondrial complex (MC) at the free cell surface (FCS). Note numerous dense cored particles. 15. EJ2 in cross section exhibiting actively synthesizing secretory epithelium centering around large nucleus. Note numerous Golgi body (GB) encircling the nucleus and finger like configuration (FC) of rER and numerous secretory materials (SM). 16. EJ2 apical region in cross section showing apocrine secretion (AS) accumulated at the free cell surface and being released by breaking open plasma membrane at the free cell surface. Note prominent Golgi bodies (GB) surrounding the nucleus. 17. Light micrograph from thick section of EJ2 exhibiting distinct muscle layers (ML) and secretory material (SM) filled lumen (L). (EP, Epithelium). 18. EJ2 secretory epithelium in cross section, characteristically exhibits concentric whorl or finger like configuration of rER surrounded by Golgi body (GB). 19. EJ2 secretory epithelium in cross section showing the formation of secretory vesicles (SV) and packaging of secretory materials. 20. EJ2 epithelium in cross section showing characteristic multivesicular body (MVb). 21. EJ2 epithelium in cross section showing numerous mitochondria (M), Golgi body (GB) and dense cored vesicles and abundant rER. (Ly, Lysed vesicles). 22. EJ2 exhibiting apocrine secretory cell and holocrine secretory cell. 23. EJ2 exhibiting the holocrine secretory cell containing multivesicular body. Note the apocrine secretory cells are distinctly marked and holocrine degenerating epithelium (HdEp) containing completely lysed materials. 24. EJ2 apical region in cross section showing well defined microvilli (MV), free cell surface containing accumulated apocrine secretory materials, the lumen contains secretory granules (Sg) and electron light and dense vesicles. (NM, Nuclear membrane). 25. EJ2 in cross section showing part of apocrine secretory cell, full of holocrine secretory cell and part of the lumen. Note the holocrine secretory cell containing degenerating epithelium (dEp), lysosomes, electron dense and light vesicles, mitochondria. (SM, Secretory material). 26. EJ2 lumen in cross section exhibits electron dense vesicles (EDV), medium electron dense vesicles (MEDV) in figure 28. 27. Light micrograph from thick sections distinctly showing the muscle layer, secretory materials, light vesicles (LV) and holocrine degenerating epithelium (HdEp) in figure 29. 28. EJ2 lumen in cross section exhibits multivesicular bodies (MVb) with electron dense material (Edm) surrounding it while numerous electron light vesicles centering it. Note the granular secretions (gs) in the lumen. 29. Light micrograph from thick section distinctly showing two different secretory cell types.

Antibiotic effects of resorcinol, gallic acid and phloroglucinol on *Heliothis armigera* Hubner (Insecta: Noctuidae)

T N ANANTHAKRISHNAN, R SENRAYAN, R S ANNADURAI and
S MURUGESAN

Entomology Research Institute, Loyola College, Madras 600 034, India

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Abstract. The antibiotic effects of 3 phenolic substances viz. resorcinol, gallic acid and phloroglucinol on the cotton bollworm *Heliothis armigera* Hubner were evaluated with particular reference to the feeding regimes, survival and pupation. Some of the characteristic antibiotic effects observed for resorcinol and phloroglucinol include reduction in consumption index and approximate digestability, increased efficiency of conversion of ingested food and efficiency of conversion of digested food, extended larval duration and high mortality. However, there was no alteration in the various food utilization efficiencies in the case of gallic acid treated individuals. The defensive response of the larvae to these toxic substances seems to be excessive defaecation. Frass analysis through thin-layer chromatography and ultraviolet characterisation indicated the presence of the ingested antibiotics, opening further avenues of investigation on the possible role of these antibiotics present in the frass in biological control.

Keywords. Antibiotic effects; *Heliothis armigera*; resorcinol; gallic acid; phloroglucinol; frass analysis.

1. Introduction

In the recent past much attention has been focussed on the chemical basis of insect-host specificity and host plant resistance (Beck 1965; Dethier 1970; Chapman 1974; Schoonhoven 1972; Gallun *et al* 1975; Hanover 1975; Kogan 1975). Most such attempts have been concentrated on chemical substances which create immediate response involving such behavioural parameters as feeding stimulants and feeding incidents (Beck and Stauffer 1957; Beck 1956, 1957, 1960). Several chemicals have been found to be responsible for the non-preference mechanism in resistant plant varieties through non-preference for oviposition and allied activities for successful establishment of an insect species on a crop (Pathak and Dale 1983). Besides this, the presence of extraneous chemicals of the host plants also prevent higher damage to the crop as has been reported in the past (Maxwell *et al* 1972; Lukefahr *et al* 1965, 1971). However, all these effects tend to be of short term duration being unable to withstand the changing pest status, which is basically different from the chronic effects of feeding, growth and survival offered by a number of chemical substances (Matsumoto 1962).

Very little work has been attempted on the chronic effects of allelochemicals on different insect groups. Most investigators (Lukefahr and Martin 1966; Williams *et al* 1980; Wiseman *et al* 1976; Waiss *et al* 1979) have reported the antibiosis process influencing insect-plant interactions particularly to *Heliothis* spp. With special reference to such chemicals as gossypol, 2-tridecanone, d-tomatine etc. several investigators (Beck 1957, 1960; Beck and Stauffer 1957; Harley and Thorsteinson 1967; Todd *et al* 1971; Feeny 1968; Reese and Beck 1976a, b, c) studied the effects of chronic ingestion of certain allelochemicals on growth, survival

and pupation. Fraenkel (1969) explained the possible role of allelochemicals on the feeding activity of herbivorous insects. Several other workers (Shaver *et al* 1970; Soo Hoo and Fraenkel 1966; Erickson and Feeny 1974) demonstrated the role of several plant allelochemicals on the feeding activity of herbivorous insects.

The present paper attempts to highlight the antibiotic effect of allelochemicals with special reference to resorcinol (1,3-dihydroxybenzene), gallic acid (3,4,5-trihydroxybenzoic acid) and phloroglucinol (1,3,5-trihydroxybenzene) on the feeding regimes, survival and pupation of *H. armigera*. Most of the resistant crop varieties screened for the preference of *Heliothis* exhibit the presence of such phenolic substances (Annadurai *et al* 1989) which provide indepth information in understanding the relationship between chemical defenses of host plants and their biological activity against insect pests.

2. Materials and methods

2.1 Stock culture

H. armigera culture was maintained in the laboratory using artificial diet following the methods slightly modified from Singh (1971) and Callahan (1962). Adults were kept in cages (30 × 30 cm) and the females oviposited on *Cicer arietinum* potted plants as well as on *Gossypium hirsutum* (MCU 5) twigs bearing buds and flowers. Eggs were treated with 0.1% formaldehyde by dipping the plants and air dried. The larvae were reared with *C. arietinum* plants for the first 4 days and subsequently transferred to artificial diet in individual culture vials (5 × 3 cm). Pieces of the diet were placed inside the culture vials for stock culture. Distilled water was used in diets and wherever water came into contact with the insects. Contamination was kept to a minimum by autoclaving the glassware at 100°C for a few hours. Containers were also washed in 0.5% formaldehyde. The larvae were reared at 29 ± 2°C under 12D:12L photoperiodic regime. The pupae were placed in a netlon mesh cage (30 × 30 cm).

2.2 Experimental cultures

Authentic phenolic substances (Sigma chemical Co., USA) were incorporated with the standard diet at concentrations of 2.48×10^{-4} M through 10^{-1} M. Similarly, for gallic acid and phloroglucinol the concentrations used were 4.23×10^{-1} through 10^{-4} and 3.65×10^{-4} through 10^{-1} M respectively. All the chemicals to be tested were dissolved in water and appropriate amounts of the solution were incorporated with the ingredients of the standard diet and homogenized. The diet was kept open for 24 h and the larvae were introduced. The appropriate controls were used for these experiments with distilled water. Each treatment consisted of 10 replicates and all the larvae were screened for survival at 10 days feeding period, 10 days pupation and survival.

2.3 Nutritional parameters

In order to have an understanding of the role of allelochemicals on weight gain, the various nutritional indices (Waldbauer 1964, 1968) were calculated. Fifth or sixth day

old larvae of 3rd instar from the stock culture were weighed and placed individually on weighed containers containing standard as well as experimental diets. The size of the diet was such that the control larvae would eat a large part of the diet given to them, but none of the larvae were in danger of running out of food before the end of the experiment. Faecal pellets were carefully separated from the left over diet piece weighed and dried to constant weight. Feeding experiments were conducted for 10 days larval period starting from sixth day. Final feeding data on 15th or 16th day of larval life, were avoided (the period during which the larvae masticate the diet for preparing pupal cells). All weights were taken on a Yamato analytical balance to the nearest accuracy of 1 mg.

2.4 Frass analysis

Thin layer chromatography (TLC) was performed to separate the phenolic compounds both from the host tissues and faecal material in the respective experiments. The R_f values and spectral ranges (UV Spectrophotometer, Hitachi 150-20) of standard phenols and phenolic acids were compared with the eluted fractions (Harborne and Williams 1969).

3. Results

Resorcinol is a substance most abundant as a compound as umbelliferone (Robinson 1963) and is a frequent constituent of more complex plant compounds (Onslow 1923). Phloroglucinol is structurally related to resorcinol and found free or in a combined state in plants (Doby 1965; Whittaker 1970), while gallic acid is a hydrolytic product of tannins and is one of the most common phenolic derivatives in tannins (Robinson 1963; Wong 1973) (figure 1).

3.1 Resorcinol

An analysis of the food consumption and the relative influence on nutritional indices indicated clear variation when fed on diets mixed with resorcinol, gallic acid

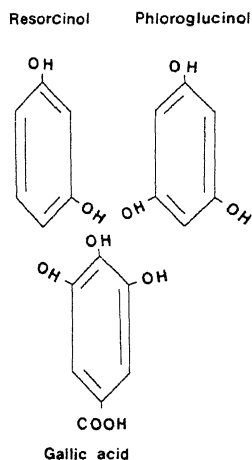


Figure 1. Chemical structure of the antibiotic substances.

and phloroglucinol. Nutritional indices were estimated for diet mixed with resorcinol and the results showed that larvae of *H. armigera* ingested only 25% of food ingested by control larvae at higher concentrations and the rate of ingestion increased at the lower concentrations. A closer look on the weight gain of the larvae also showed that larvae fed on the incorporated diet had a poor weight gain as compared to control (table 1). Larvae fed on the phenol incorporated diets excreted excess faecal pellets when compared to normal individuals presumably in an act to egest the toxic substances in the diet. The consumption index (CI) as well as approximate digestability (AD) showed similar values being higher in control and considerably reduced in treated individuals. The values of efficiency of conversion of digested food (ECD) doubled in higher concentrations of resorcinol treated diets when compared to control and the values showed considerable reductions at moderate and lower concentrations. The efficiency of conversion of ingested food (ECI) showed higher values in the case of controls and relatively lesser ECI values were recorded in treatments. The higher ECD and lower ECI values in treatments compared to controls could presumably be attributed to the fact that the larvae defaecate excessively the diet containing toxic substance in a physiological event to remove the toxic substance and hence relatively less quantity of food material is allocated to body tissue.

A comparison of the larval duration and larval weight clearly indicated that the larvae fed on resorcinol significantly extended their larval duration with a significant difference in their body size (table 2). Among the various treatments, 2.75×10^{-1} concentration increased the larval duration several folds compared to control. The mean pupal weight also varied among treatments and the size being very small in higher concentrations. An overall analysis of larval survival showed that 38% of larval mortality occurred in higher concentrations of resorcinol and larval mortality decreased considerably at lower concentrations. Considerable pupal mortality was also observed among resorcinol-fed individuals especially at higher concentrations (table 2). Behavioural as well as feeding activities of the treated larvae confirmed the hypothesis that most of the larval mortality and prolonged larval duration are largely attributed to the inhibition of ingestion of food as well as disproportionate defaecation during larval stages. Several larvae were also noticed in a struggling condition to remove their larval exuviae during the moulting process.

3.2 Gallic acid

A totally different picture was obtained when individuals of *H. armigera* were subjected to diet with gallic acid. The nutritional indices and related parameters showed that almost similar quantity of diet was ingested as that of control at higher concentrations of gallic acid, the quantities being 93.6, 66.3, 63.7 and 76.3% of that of control when fed on concentrations 4.73×10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} respectively. On the other hand the amount of faeces ejected was greater in treatments compared to control and the values being 344.4, 255.8, 244.3 and 185.3% at various concentrations indicated in table 3. The experimental larvae assimilated a much smaller proportion of their food (AD). The efficiency of conversion of assimilated food (ECD) was much lower at all concentrations compared to control. The inhibition of both these processes caused a marked reduction in the overall efficiency of ingested food (ECI) (table 3).

Table 1. Effect of resorcinol on nutritional indices and related parameters of *H. armigera*.

Parameters	Parameters (% of control)						CD value at 5%
	Control	2.75×10^{-1} M	2.75×10^{-2} M	2.75×10^{-3} M	2.75×10^{-4} M		
Average food consumed/day (mg)	330.3	025.0	042.9	059.3	064.5	31.96	
Final wt. gain (mg)	118.3	028.4	042.0	034.8	050.9	16.46	
Average wet wt. of faeces/day (mg)	065.5	089.0	203.2	213.1	148.9	20.57	
CI	001.6	043.8	068.8	075.0	112.5	00.261	
AD	076.2	048.6	045.0	053.1	061.3	08.59	
ECD	039.4	171.3	133.5	112.7	110.4	08.18	
ECI	025.4	088.9	074.4	047.2	060.2	05.04	

Values are mean of 10 replicates.

Table 2. Effect of resorcinol on larval duration, pupation and survival of *H. armigera*.

Parameters	Treatments						CD value at 5%
	Control	2.75×10^{-1} M	2.75×10^{-2} M	2.75×10^{-3} M	2.75×10^{-4} M		
Larval duration (days)	014.8	022.4	021.4	021.4	018.3	2.04	
Mean larval growth prior to pupation (mg)	362.5	169.2	224.6	236.8	243.6	—	
Mean pupal wt. (3 days after pupation) (mg)	302.4	132.6	172.3	204.2	231.8	—	
Percentage larval survival (at the end of 5th larval instar)	100.0	62.0	71.0	70.0	80.0	—	
Percentage pupal mortality	—	23.0	26.0	7.0	3.0	—	

Values are mean of 10 replicates.

Table 3. Effect of gallic acid on nutritional indices and related parameters of *H. armigera*.

Parameters	Control	Parameters (% of control)					CD value at 5%
		4.23×10^{-1} M	4.23×10^{-2} M	4.23×10^{-3} M	4.23×10^{-4} M		
Average food consumed/day (mg)	330.3	93.6	66.3	63.7	76.3	37.48	
Final wt. gain (mg)	118.3	08.5	07.5	19.2	34.3	09.35	
Average wet wt. of faeces/day (mg)	065.5	344.4	265.8	244.3	085.2	32.36	
CI	001.6	181.3	131.3	156.3	150.0	00.39	
AD	076.2	029.9	031.5	036.4	059.7	07.76	
ECD	039.2	059.0	039.9	083.2	082.7	06.21	
ECI	025.4	014.9	017.3	046.9	074.4	02.78	

Values are mean of 10 replicates.

Table 4. Effect of gallic acid on larval duration, pupation and survival of *H. armigera*.

Parameters	Control	Treatments					CD value at 5%
		4.23×10^{-1} M	4.23×10^{-2} M	4.23×10^{-3} M	4.23×10^{-4} M		
Larval duration (in days)	014.8	020.5	020.2	019.4	018.9	12.18	
Mean larval growth prior to pupation (mg)	362.5	103.2	122.4	116.8	143.8	—	
Mean pupal wt. (3 days after pupation) (mg)	302.4	092.2	098.2	108.2	114.6	—	
Percentage larval survival (at the end of 5th larval instar)	100.0	40.0	46.0	48.0	65.0	—	
Percentage pupal mortality	—	66.0	60.0	50.0	50.0	—	

Values are mean of 10 replicates.

Extended larval duration as well as reduction in larval and pupal size was well evident at all concentrations as compared to control. Higher larval and pupal mortality were recorded at higher concentrations of gallic acid compared to control (table 4). Behavioural observations clearly indicated that the larvae tend to feed continuously as well as defaecate excessively especially at higher concentrations of gallic acid. Further, it was also observed that larvae developed muscular lesions and convulsions in the posterior half of the body which make them totally immobile and the individuals tend to display a crawling movement.

3.3 *Phloroglucinol*

A new type of antibiotic effect was observed in the larvae fed on diet with phloroglucinol, where the larvae hardly ingest any diet. Food utilization experiments indicated that larvae fed only 27% of control diet at 3.65×10^{-1} concentration compared to control. A moderate faecal output was also recorded in all concentrations. A significant difference in the final weight gain was observed between control and various experimental individuals at different concentrations (table 5). Phloroglucinol did inhibit AD at all concentrations. ECD was reduced significantly at higher concentrations of phloroglucinol and gradual increase was noticed at lower concentrations. As a result of it, the ECI was reduced to a highly significant level by phloroglucinol at higher concentrations and a gradual increase is recorded in corresponding concentrations (table 5). Similar to other compounds, larvae fed on phloroglucinol extended their larval duration with a poor larval and pupal weight gain. High larval and pupal mortality was also evident compared to control individuals (table 6). The higher larval mortality can greatly be attributed to starvation due to inhibition of ingestion in experimental individuals compared to control. Secondly, the pupal mortality may presumably be due to low hormonal titers ultimately preventing adult emergence.

3.4 *Frass analysis*

In order to assess the defensive response of the larvae to plant allelochemicals as well as to substantiate the excretion of considerable amount of allelochemicals along with faecal pellets which tend to either attract/repel the natural enemies, the faeces of *H. armigera* when fed on different chemical regimes were subjected to TLC and the presence of allelochemicals were characterised based on UV spectral analysis (figure 2). A comparison of UV spectra of the phenolic substances (resorcinol, gallic acid and phloroglucinol) both from host plant samples as well as from faecal pellets of *H. armigera* indicated the presence of all the compounds in the faeces especially at higher concentrations confirming the hypothesis that insect herbivores can avoid toxic effects of plant allelochemicals through excessive defaecation as one possible defensive act against plant growth reducing substances. Table 7 provides the spectral ranges and R_f values of all the 3 compounds from various host tissues were analysed.

4. Discussion

The present investigation considerably substantiates the hypothesis that plant allelochemicals may have chronic effects on rate of growth, ingestion and utilization

Table 5. Effect of phloroglucinol on nutritional indices and related parameters of *H. armigera*.

Parameters	Treatments (% of control)					
	Control	3.65×10^{-1} M	3.65×10^{-2} M	3.65×10^{-3} M	3.65×10^{-4} M	CD value at 5%
Average food consumed/day (mg)	330.8	027.1	023.5	029.9	037.4	26.4
Final wt. gain (mg)	118.3	005.5	008.5	009.0	017.3	06.95
Average wt. of faeces/day (mg)	065.5	074.0	074.6	078.8	119.8	58.05
CI	001.6	076.3	075.0	091.3	112.5	00.33
AD	076.2	060.8	071.2	053.7	048.7	07.85
ECD	039.2	078.1	070.2	059.0	114.5	07.01
ECI	025.4	046.1	062.1	038.6	053.4	03.77

Values are mean of 10 replicates.

Table 6. Effect of phloroglucinol on larval duration, pupation and survival of *H. armigera*.

Parameter	Treatments					
	Control	3.65×10^{-1} M	3.65×10^{-2} M	3.65×10^{-3} M	3.65×10^{-4} M	CD value at 5%
Larval duration (in days)	014.3	021.9	020.4	019.8	019.4	12.13
Mean larval growth prior to pupation (mg)	362.5	096.4	103.2	102.2	139.8	—
Mean pupal wt. (3 days after pupation) (mg)	302.4	083.8	092.6	088.6	106.6	—
Percentage larval survival (at the end of 5th larval instar)	100.0	034.0	040.0	040.0	060.0	—
Percentage pupal mortality	—	060.0	060.0	050.0	050.0	—

Values are mean of 10 replicates.

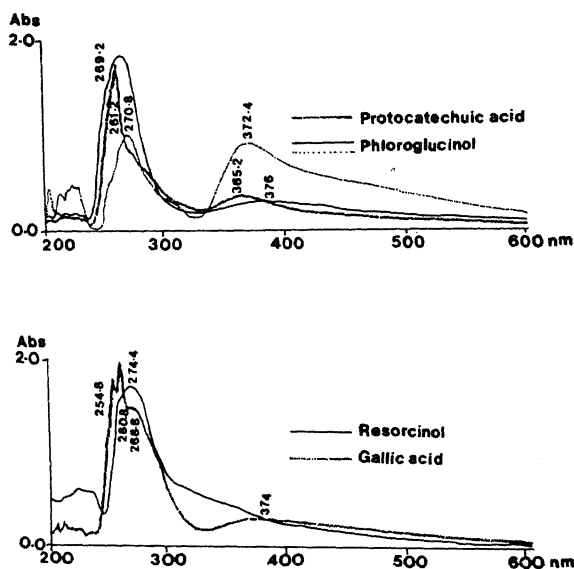


Figure 2. UV spectra of the antibiotic phenolic substances separated from the frass of *H. armigera* fed on different host tissues as well as individuals fed on phenol incorporated semi-synthetic diet.

of food by herbivores (Whittaker 1970). Evidences in support of this phenomenon have been indicated by Beck and Reese (1976) and Reese and Beck (1976a, b, c) on black cutworm, *Agrotis ipsilon*. Nutritional index experiments showed that resorcinol did not exert much adverse effect on any of the nutritional indices, but still tend to affect growth especially at higher concentrations. Added to this, resorcinol inhibited normal growth, but did not inhibit the larvae from pupation and thus it appears that resorcinol might exhibit growth inhibition mainly through inhibition of ingestion (Reese and Beck 1976a, b, c). The increased survival percentage of larvae as well as pupae when fed on resorcinol among all compounds may also be attributed to large scale excretion of resorcinol along with faeces. Unlike resorcinol, gallic acid inhibited growth by primarily reducing the efficiency of conversion of assimilated food and inhibition of the ECI. Gallic acid also reduced survival and pupation presumably through its action against its digestive enzymes and gut cells in the hind gut. This was well evident in *H. armigera* by exhibiting continuous defaecation and muscular lesions of hind gut and inactivity of the larvae. Dissected individuals also revealed the fact that a large amount of faecal material accumulate in the hindgut making the larvae immobile. Similarly, phloroglucinol inhibited larval weight gain mainly through inhibition of food ingestion and affecting ECD and ECI values. The survival and pupation of *H. armigera* is severely affected when fed on phloroglucinol compared to normal individuals and resorcinol treated individuals. However, resorcinol as well as other compounds at lower concentrations did not show much impact on larval survival and pupation compared to higher concentrations. The apparent stimulation of growth, survival and pupation at low concentrations of certain plant compounds may have been due to hormoligosis, wherein the harmful substances of stress agents may stimulate growth etc. (Luckey 1968). Similar reports of enhancing the growth and survival

Table 7. List of phenolic substances characterised from the host tissues and frass of *H. armigera*.

Host plants	Phenols identified from host plant tissues	Phenols recovered from frass	Solvent system (mobile phase)	R_f value ($\times 100$)	Spectral maxima (nm)
<i>G. hirsutum</i>					
Bolls	Phloroglucinol	Benzene derivatives	HOAc-CHCl ₃	19	274.4
Young leaves	Protocatechuic acid	Resorcinol			
Mature leaves	Phloroglucinol	Phloroglucinol	Benzene-MeOH-HOAc	12	269.2 270.8
Senescent leaves	Resorcinol				
<i>C. cajan</i>					
Pod	Phloroglucinol	Benzoic acid derivatives	HOAc-CHCl ₃	07	260.8
Young leaves	Protocatechuic acid	Gallic acid			
Senescent leaves	Resorcinol	Protocatechuic acid (3,4-dihydroxybenzoic acid)	Benzene-MeOH-HOAc	18	261.2
<i>A. esculentus</i>					
Pod					
Young, mature and senescent leaves	Protocatechuic acid				

of *A. ipsilon* at lower concentrations of phloroglucinol was reported by Reese and Beck (1976a, b, c). The poor rate of ingestion of diet with phloroglucinol may be attributed to the direct action on the chemoreceptory system of the larvae as described earlier in *A. ipsilon* by Reese and Carlson (1974). The reduction in growth, survival and pupation of *H. armigera* may also be the result of chronic effects of lower assimilation and efficiency of conversion of food. In the present investigation, although the larvae consume thrice the quantity of diet mixed with gallic acid compared to control, growth and survival are still affected in *H. armigera* in a greater way. Erickson and Feeny (1974) similarly found that sinigrin did not inhibit ingestion, but reduced assimilation of food by larvae of *Papilio polyxenes asterius* Stoll. It was observed that unlike gallic acid and sinigrin, phloroglucinol and resorcinol affected ingestion thereby affecting larval growth and survival.

The other possible factor related to the lower survival and reduced growth of *H. armigera* in various treatments of resorcinol, gallic acid and phloroglucinol is apparently due to the change in the digestive enzymes or membrane proteins of the microvilli in the gut, as a result altering the digestion and assimilation efficiencies of the larvae. Reese and Beck (1976a, b, c) hypothesized that *p*-benzoquinone may change the sulphhydryl-disulphide equilibrium of important digestive enzymes and membrane proteins of the microvilli in the gut. The possible other mode of action of this compound on both enzyme and hormonal regulation of the larvae which may also play a major role in the larval survival and growth requires greater attention. In the present study, gallic acid and phloroglucinol is found to be more effective compared to resorcinol which can largely be attributed to the differential nature of gut pH facilitating the action of the concerned compound. Goldstein and Swain (1965) reported that a high gut pH is suggested as a mechanism of defence against tannins, since it would inhibit complexing of tannins with proteins. Higher faecal output in the case of *H. armigera* individuals fed on gallic acid may also act as a defensive strategy of the larvae to avoid toxicity. Studies of Self *et al* (1964) supported the present view, wherein they have indicated that excretion and egestion of intact nicotine along with frass is an adaptive mechanism in the tobacco hornworm *Protoparce sexta* (John) when fed on tobacco. Convulsion and immobility are the injurious effects of gallic acid on the gut as well as posterior muscles of the larvae. Bernays (1978) observed that *Locusta migratoria* fed on diet with tannic acid showed midgut and caecum lesions associated with gut staining and broken gut epithelial cells.

Several other reports of antibiotic activity of several allelochemicals exist in plants on the survival and growth of Lepidopterans. Sesamin was found to be a growth inhibitor for silk worm, *Bombyx mori* L. (Kamikado *et al* 1975). Chan *et al* (1978) reported that the condensed tannins have higher antibiotic activity for *H. virescens*. Similarly gossypol has been implicated as the primary substance responsible for the cotton plant resistance to several pests (Bottger and Patana 1966; Shaver *et al* 1970; Bell 1974). Meanwhile, several phenolic compounds are important in several respects during the development of *B. mori* (Kato 1978).

It is also a well known fact that several allelochemicals of plant origin not only affect the survival and growth of the herbivores, but also make them available to natural enemies for a longer period and raises the probability of mortality. Therefore, the extended larval period and poor growth rate of *H. armigera* make

themselves available to an array of natural enemies in a natural ecosystem. Feeny (1976) indicated that plants with digestibility reducing substances (DRS), support herbivores that are more heavily attacked by natural enemies. The foregoing results therefore, clearly indicate the antibiotic effects of 3 allelochemicals viz. resorcinol, gallic acid and phloroglucinol which largely affect the nutritional indices, survival and growth in addition to morphological malformations. Though an evidence of clear role of antibiotics in growth inhibition and survival of *H. armigera* is highlighted, the host plant tissues rich in these compounds at appropriate concentrations may impose precise mechanisms of non-preference to herbivores, which warrants a deeper analysis of the problem.

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In vivo* effect of dimethoate on acetylcholinesterases from a freshwater teleost, *Notopterus notopterus

R S HANDE and P V PRADHAN

Department of Biological Sciences, R J College, Ghatkopar, Bombay 400 086, India

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Abstract. The effect of exposure to dimethoate, an organophosphorus insecticide, on the activity of soluble and membrane-bound fractions of acetylcholinesterases from the brain of the Indian razor-fish, *Notopterus notopterus* was studied. The fishes were exposed to a concentration of 2 ppm of the insecticide for a period of 4 weeks. The two fractions of the enzyme exhibited a reduced efficiency, as indicated by V_{\max}/K_m ratios. The exposure to the insecticide resulted in 96.25% reduction in V_{\max}/K_m for the membrane-bound fraction and 68.7% reduction in the corresponding value for the soluble enzyme as compared to those of the control set of fishes.

Keywords. Dimethoate; acetylcholinesterases; *Notopterus*.

1. Introduction

The organophosphorus compounds are well-known anticholinesterase substances, many of which are used as effective insecticides (Gage 1953; Murphy 1966). Although beneficial in protecting the crop against insect pests, these pesticides have posed a grave environmental problem because of their indiscriminate use in fields. It is a well-known fact that the organophosphates, being often soluble in water adversely affect the non-target species, particularly the aquatic fauna. Some workers (Coppage 1972; Macek *et al* 1972) have suggested that the measurement of acetylcholinesterase (AChE) activity from the aquatic environment gave an indication of the extent of organophosphate pollution in that environment.

Dimethoate [0-0-dimethyl S-(N-methylcarbamoyl)methyl phosphorodithioate], an organophosphorus insecticide, is popular because of its high insecticidal and low mammalian toxicity (Dubale and Awasthi 1980). There are a few reports on the histopathological lesions caused by dimethoate in fishes like *Channa gachua* (Dalella *et al* 1978), *Heteropneustes fossilis* (Dubale and Awasthi 1980, 1982) and *Barbus stigma* (Singh and Karpagaganapathy 1988) and consequent variations in the activities of enzymes like alkaline phosphatase and succinate dehydrogenase. Recently Santhakumari and Pradhan (1988) reported an inhibition of AChE from the brain of *Haplochilus lineatus* by Rogor.

The present investigation was undertaken to study the effect of dimethoate, a synthetic organophosphate insecticide widely used in India, on AChE from the brain of the razor-fish, *Notopterus notopterus*, a commercially important edible fish found in freshwater masses throughout India, where it is likely to be exposed to various pesticides particularly in the ponds and lakes in the vicinity of agricultural fields.

2. Materials and methods

The fishes were procured from a local freshwater lake and maintained in well

aerated laboratory aquaria for 2 weeks to acclimate them to the laboratory conditions. Fishes were kept in 6 sets of 5 each in 40 litres capacity glass aquaria containing 30 litres of dechlorinated tap water and were fed on live *tubifex* and *Daphnia* daily. All fishes were in a length group of 14–18 cm weighing 58–82 g. Three sets of fishes (total 15) were exposed to a concentration of 2 ppm of dimethoate (technical 95–98%) for a period of 4 weeks. An equal number of fishes (the other 3 sets) were maintained as control. The water from the experimental sets was replaced on every alternate day by water containing same quantity of pesticide. No mortality was recorded in any set of fishes during the period of experiment. Fishes were sacrificed at the end of the experimental period and the brains were quickly dissected out and washed with chilled 0.9% NaCl with 0.1% EDTA. The brains from the experimental set of fishes were pooled together and the same was done with the control sets. The enzyme was extracted by the method of Varela (1973) and assayed by the method of Ellman *et al* (1961) using acetylthiocholine iodide (ATChI) as the substrate in a Beckman spectrophotometer. The specific activity was expressed as Δ absorbance/0.1 mg protein/min. Proteins were estimated by the method of Lowry *et al* (1951) using bovine serum albumin fraction V from Sigma Chemical Co., USA as standard.

3. Results and discussion

The initial experiments were carried out to establish the basic parameters like pH, time, temperature, protein and DTNB concentrations. The effect of varying concentrations of substrates (0.1–3 mM) on the enzyme activity was estimated at optimum parameters like pH 8.5, 22°C and 0.4 mM DTNB. Enzyme extracts corresponding to 0.1 and 0.15 mg of proteins were used for the soluble and the membrane-bound enzyme assays respectively. The substrate saturation curves were plotted for both enzyme fractions from the brain of the control and the insecticide-exposed (experimental) set of fishes (figure 1). The data obtained were

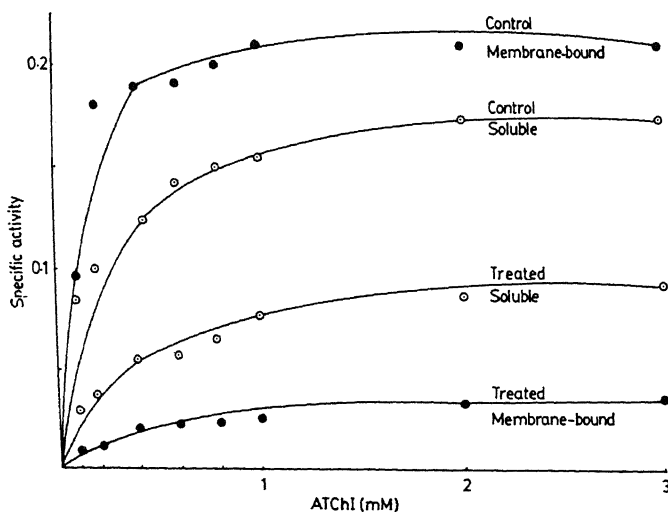


Figure 1. Substrate saturation curves for the soluble and the membrane-bound AChE from the brain of control and dimethoate-exposed *N. notopterus*.

also analysed by the direct plots of Eisenthal and Cornish-Bowden (1974). The kinetic parameters computed by these plots are summarised in table 1. In control set of fishes, the membrane-bound AChE is distinctly more active, as indicated by low K_m and high V_{max} values, than the soluble fraction of the enzyme.

Table 2 presents a comparison of the effect of dimethoate exposure on the soluble and the membrane-bound AChE and the resultant variation in K_m , V_{max} and V_{max}/K_m ratios. It is evident from table 2 that the exposure to dimethoate in *N. notopterus* results in a significant inhibition of the activity of the soluble as well as the membrane-bound fractions of AChE, however this is more pronounced in the latter (−86.3%) as compared to the former (−51.91%). It also results in an overall

Table 1. Values of K_m and V_{max} computed by different methods of analysis for the soluble and the membrane-bound AChE from the brain of control and dimethoate-exposed *N. notopterus*.

	K_m (mM of ATChI)		V_{max} (absorbance/0.1 mg protein/min)	
	MM	ECB	MM	ECB
Soluble AChE				
Control	0.112	0.150 ± 0.012 (n = 23)	0.172	0.173 ± 0.0041 (n = 23)
Exp.	0.252	0.235 ± 0.003 (n = 13)	0.093	0.083 ± 0.001 (n = 13)
Membrane-bound AChE				
Control	0.1	0.113 ± 0.003 (n = 15)	0.232	0.251 ± 0.009 (n = 15)
Exp.	0.4	0.412 ± 0.0046 (n = 18)	0.033	0.0344 ± 0.0048 (n = 18)

± Values expressed as SE.

MM, Michaelis-Menten plot.

ECB, Eisenthal and Cornish-Bowden plot.

Table 2. Effect of dimethoate exposure on the activity of the soluble and the membrane-bound AChE from the brain of control and experimental *N. notopterus*.

	Soluble AChE			Membrane-bound AChE		
	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
Control						
MM	0.172	0.112	1.535	0.232	0.100	2.32
ECB	0.173	0.150	1.153	0.251	0.113	2.22
Exp.						
MM	0.093	0.252	0.364	0.033	0.400	0.082
ECB	0.083	0.235	0.350	0.034	0.412	0.083
Variation (%)						
MM	125.0	−45.93	−75.88	−85.77	300.0	−96.46
ECB	156.6	−51.91	−68.70	−86.3	364.6	−96.25

V_{max} , Expressed as Δ absorbance/0.1 mg protein/min.

K_m , Expressed as mM of ATChI.

MM, Michaelis-Menten plot.

ECB, Eisenthal and Cornish-Bowden plot.

decrease in the efficiency of the enzyme fractions, as indicated by the lower V_{\max}/K_m ratios. However the two fractions of the enzyme respond differentially—the V_{\max}/K_m ratio for the membrane-bound fraction being reduced by 96.25% as compared to 68.7% reduction in the V_{\max}/K_m ratio for the soluble fraction. Such an observation was earlier reported by Santhakumari and Pradhan (1988), while studying the effect of dimethoate on AChE from the brain of *Haplochilus lineatus*.

The role of membrane-bound AChE in synaptic transmission and neuromuscular functions in vertebrates have well been established, however, the role of soluble AChE has not yet been clearly defined. The most plausible role of the soluble AChE is its involvement in the energy metabolism of nerve cells by controlling the availability of free acetyl groups as suggested by Varela (1975). The inhibitory effect of dimethoate on the membrane-bound as well as the soluble fractions of AChE indicates that this organophosphate insecticide not only affects the neuromuscular functions but may also interfere in vital processes like energy metabolism of nerve cells etc.

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Biochemical aspect of varietal resistance to rice green leafhoppers, *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stål)

KASI VISWANATHAN and M B KALODE

Department of Entomology, Directorate of Rice Research (AICRIP), Hyderabad 500 030, India

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Abstract. Biochemical analysis of 4 rice varieties and a weed showed that the total free amino acid content was greater in the susceptible variety TN 1 than in Ptb 2, Ptb 7 and Ptb 18 which are resistant to both the species of green leafhoppers. However, the weed *Leersia hexandra* the most suitable host of *Nephotettix nigropictus*, had the lowest amount of free amino acids. No apparent differences were observed in relation to sucrose, glucose and fructose content in the test varieties. Total phenol content was the highest in *Leersia hexandra* followed by resistant varieties, while it was the lowest in the susceptible rice variety.

Bioassay of plant extracts in various organic solvents showed that the chloroform and acetone extracts of the resistant variety Ptb 18 were phagodeterrents to either species of green leafhoppers, while the extract of the susceptible variety with the same solvent was phagostimulant. Of the various chemicals bioassayed, sucrose (5%) among the sugars tested, was found to be highly stimulatory for feeding. Among amino acids, serine, alanine and glutamine were feeding stimulatory in nature. On the other hand, most of the amino acid derivatives, all organic acids and phenolic compounds tested exhibited phagodeterreny.

Keywords. Varietal resistance; *Nephotettix* spp.; biochemical resistance; rice; leafhoppers.

1. Introduction

The rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål) form the predominant species causing severe plant injury through direct feeding and acting as vectors for tungro virus disease. In earlier communication, we have reported screening of 108 rice varieties and identification of highly resistant cultivars for both the species (Viswanathan and Kalode 1984). In the follow up intensive host range studies, it was observed that *N. virescens* had a narrow preference for susceptible rice varieties alone while *N. nigropictus* had a wider host range (Viswanathan and Kalode 1986). Further, it was suggested that host selection by these two species of green leafhoppers was of chemosensory nature and principally by gustatory stimuli (Viswanathan and Kalode 1987).

In most cases host plant resistance is biochemical in nature and phytochemicals involved are mostly groups like acetogenins, alkaloids, flavonoids, glycosides, isoprenoids etc. (Pathak and Dale 1983). These chemicals act as feeding deterrents, growth inhibitors, toxicants and alike. Besides such secondary plant substances, certain principal nutrients contribute towards susceptibility of host plants. While information is available on performance of certain chemicals acting as probing/sucking stimulants/deterrents against the brown planthopper, *Nilaparvata lugens* (Sogawa 1982), no detailed studies have been made in relation to green leafhoppers. In this paper we report results of analysis of susceptible and resistant rice varieties for certain important biochemicals and bioassay of plant extracts and

various chemicals for feeding response by the two species of green leafhoppers in an effort to underline the biochemical basis of varietal resistance.

2. Materials and methods

Based on varietal resistance and host specificity studies (Viswanathan and Kalode 1986), 4 rice varieties viz. Ptb 2, Ptb 7, Ptb 18 (highly resistant), TN 1 (susceptible) and a weed *Leersia hexandra* (showing differential reaction) were included for study in the present investigation.

2.1 Biochemical analysis

Leaf material of the resistant and susceptible varieties was analysed for free amino acids, sugars and total phenol content adopting standard analytical methods (Joslyn 1970).

Quantitative estimation of amino acids was made using an automatic amino acid analyser. Sucrose, glucose and fructose were estimated qualitatively by unidimensional ascending paper chromatography using phenol:water (4:1 v/v). Total phenol was estimated colorimetrically after ethanol extraction using Folin-Ciocalteu's reagent.

2.2 Bioassay of plant extracts and authentic chemicals

2.2a Plant extracts: Rice varieties TN 1 and Ptb 18 and the weed *L. hexandra* were used. A known quantity of leaves to be extracted were ground with little quantity of water in a glass mortar. Organic solvents viz. acetone, ethyl alcohol, ethyl ether, benzene and chloroform were used for extraction. The ground tissue was made up with the solvent to represent 8% of the solvent. Solvent fractions were collected and dried in a rotary vacuum evaporator. The residue was dissolved in distilled water to represent 2.5 ml of water for every gram of tissue. Response of the green leafhoppers to each plant extract was measured in terms of number of stylet probings made during the test period, using an apparatus as shown in figure 1. It consisted of glass tubes of 2 cm diameter and 5 cm long and opened at both ends. A square parafilm membrane was stretched and fixed on the open end of one of the tubes. About 1 ml of the test solution was placed on this membrane and covered with a similar piece of membrane. The other glass tube used for caging test insects was attached to the tube with small strip of parafilm membrane. One female and one male adult insects were caged for 3 h in upper chamber. At the end of this period stylet sheaths formed in parafilm were stained using 1% erythrosin. The number of feeding marks were counted under a binocular microscope (figure 2). Positive or negative percentage values of feeding stimulation were calculated considering 5% sucrose as 100% feeding stimulant. Higher proportion of feeding marks was treated as feeding deterrent response while lesser proportion of feeding marks as feeding stimulant response.

2.2b Authentic chemicals: Twenty amino acids, 5 sugars, 4 organic acids, 7 phenolic compounds and 48 available amino acid derivatives were bioassayed as



Figure 1. The glass tube set-up for bioassaying various chemicals and plant extracts for feeding response of *Nephotettix* spp.

per the technique described in previous experiment and relative feeding response was determined. Amino acids (1%), sugars (1 and 5%) and other chemicals (organic acids, phenolic compounds and amino acid derivatives at 0.5%) were tested as solutions. In other experiment, amino acids and other chemicals were tested in combination with 5% sucrose at the above concentrations.

3. Results and discussion

3.1 Biochemical analysis

3.1a Amino acid content: An analysis of the plant material showed that among the 17 amino acids detected, all the individual amino acids except glycine and proline were in higher concentrations in susceptible variety TN 1 than in test varieties of rice or the weed *L. hexandra* (table 1). Resistant varieties viz. Ptb 2, Ptb 7 and Ptb 18 had lesser amino acid content ($107\text{--}117.3\text{ }\mu\text{g}/100\text{ g}$) as compared to susceptible variety ($172.3\text{ }\mu\text{g}/100\text{ g}$). However, *L. hexandra*, the susceptible host of *N. nigropictus* had the lowest quantity of amino acids ($22.7\text{ }\mu\text{g}/100\text{ g}$) indicating

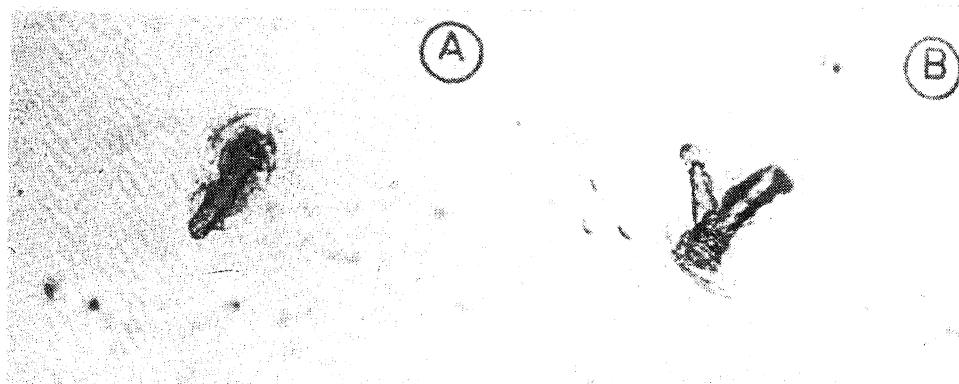


Figure 2. Stylet sheaths made by *Nephrotettix* spp. on parafilm membrane. (A) Single. (B) Bifurcated.

Table 1. Free amino acid contents in selected rice varieties and the weed *L. hexandra*.

Amino acids	$\mu\text{g}/100 \text{ g}$ of leaf material				
	Rice varieties				<i>L. hexandra</i>
	TN 1	Ptb 2	Ptb 7	Ptb 18	
Lysine	2.5	1.5	1.3	1.5	0.3
Histidine	0.7	0.6	0.5	0.5	0.4
Arginine	3.8	2.0	1.4	1.7	0.3
Aspartic acid	8.3	6.7	11.0	3.9	1.3
Serine	41.4	14.7	13.7	19.9	—
Threonine	—	—	—	—	1.1
Glutamic acid	59.8	47.2	36.0	55.9	11.0
Proline	1.5	1.7	16.3	2.2	0.3
Glycine	1.5	2.0	0.7	3.0	0.3
Alanine	19.5	14.0	14.3	15.0	4.3
Cystine	—	—	—	—	—
Valine	2.1	1.4	1.4	0.9	—
Methionine	—	—	—	—	0.7
Isoleucine	27.7	13.5	19.4	12.8	0.2
Leucine	1.8	1.1	1.2	1.0	2.5
Tyrosine	0.7	0.5	—	0.5	—
Phenylalanine	1.1	0.9	—	0.5	—
Total	172.3	107.8	117.2	117.3	22.7

thereby that the amino acid content may not be a principal factor for resistance to both the species. Sogawa (1973) suggested that brown planthopper feeding was more pronounced on the rice plants containing higher concentrations of amino acids like aspartic acid, glutamic acid, asparagine and alanine which acted as feeding stimulants. Peng *et al* (1979) attributed resistance to brown planthopper in rice varieties Nan You 6 and Vei You 6 to lower contents of aspartic acid, asparagine, valine, alanine and glutamic acid which act as feeding stimulants. About 3–5 times differences in the concentrations of these amino acids between resistant

ceptible varieties were reported. Though earlier studies at the International search Institute (IRRI) implicated low concentration of asparagine as a factor for brown planthopper resistance in rice variety Mudgo (Sogawa hak 1970), later studies revealed no significant variation between brown hopper resistant and susceptible isogenic lines of rice in the concentration and tion of free amino acids (Sogawa 1982).

Sugar content: All the test rice varieties and *L. hexandra* weed had 3 sugars (glucose and fructose) almost in comparable quantities (figure 3), thereby g no significant role of these sugars in resistance against the leafhoppers. y, Sogawa and Pathak (1970) could not correlate the susceptibility (IR 8 and nd resistance (Mudgo) of rice varieties with sugar content. On the other raiah *et al* (1982) observed higher amounts of sugars in brown planthopper le varieties (Tella Hamsa and Jaya) as compared to resistant variety

Phenol content: The total phenols present were lowest in the susceptible FN 1, while the resistant varieties had higher concentrations. On the other *hexandra*, a susceptible host of *N. nigropictus* had the highest quantity of enols (figure 4). Probably, further analysis of individual phenols in test may give some clue regarding the role of such phenolic compounds in g resistance to green leafhoppers. Krishna (1977) found that phenolic nds in healthy samples of resistant and susceptible varieties were not e of any role played by these compounds but upon infestation by brown per, resistant variety was found to react sharply in producing higher of phenols. In general, resistant varieties contained more phenolic nds than susceptible varieties (Pathak and Khush 1979).

Effect of plant extracts on feeding response

s were made to extract the resistance/susceptible factor by using different solvents. Chloroform and acetone extracts of the resistant rice variety

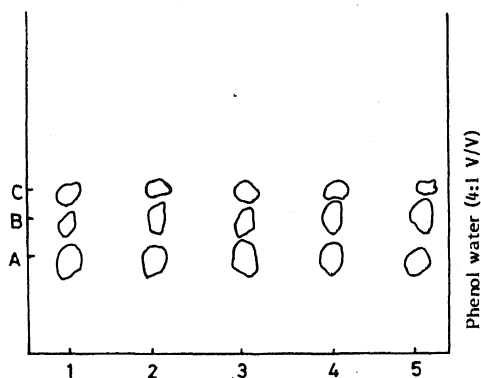


Figure 3. Paper chromatogram showing various sugars in different rice varieties. (A), Sucrose; (B), glucose; (C), fructose. (1), TN 1; (2), Ptb 2; (3), Ptb 7; (4), Ptb 18; (5), weed *L. hexandra*.

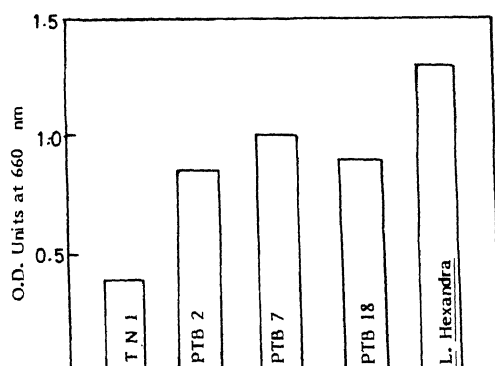


Figure 4. Relative concentrations of total phenols in leaf blades of different rice varieties and the weed *L. hexandra*.

Table 2. Effect of plant extract on the feeding behaviour of green leafhoppers *N. virescens* and *N. nigropictus*.

Leaf extract	Percentage probing marks*					
	<i>N. virescens</i>			<i>N. nigropictus</i>		
	TN 1	Ptb 18	<i>L. hexandra</i>	TN 1	Ptb 18	<i>L. hexandra</i>
Water	57.3	51.0	56.8	49.5	44.6	58.6
Acetone	13.8	25.0	28.3	17.5	29.6	26.0
Protein fraction of acetone	18.5	19.6	22.1	19.3	21.0	21.8
Ethyl alcohol	55.5	39.0	56.0	46.8	39.6	50.8
Ethyl ether	18.6	21.6	20.0	15.0	18.5	16.5
Benzene	20.6	30.0	31.3	28.3	28.3	24.8
Chloroform	11.1	22.0	28.6	13.3	29.0	18.8
CD (0.05)	4.5362					
CV (%)	13.5					

*Number of feeding marks in 5% sucrose solution was considered as 100%.

Ptb 18 and the weed *L. hexandra* showed phago-deterrency resulting in higher percentage of probing marks in the bioassay test, while TN 1 extract using the same solvents had stimulating effects against both the species (table 2). In a choice setup brown planthoppers tended to settle more in number on filter paper/cotton soaked, treated with ethanol extracts of the susceptible and resistant rice varieties. However, methanol extract of only susceptible variety TN 1 and not of resistant Mudgo evoked such response (Kalode 1971). Studies carried out at the International Rice Research Institute on the causes of brown planthopper resistance indicated that insects feeding on petroleum ether and chloroform extract of Mudgo \times IR 8 plants suffered greater mortality than those feeding on similar extracts of IR 8 plants (IRRI 1972, 1973).

3.3 Effect of authentic chemicals on feeding response

Primary nutrient chemicals and secondary plant substances have been reported to be

important phagostimulants for many oligophagous as well as polyphagous insects (Schoonhoven 1969).

3.3a Response to sugars: In the present investigation sucrose, glucose and fructose elicited greater feeding response in both the species at 5% than at 1% level. However, sucrose at 5% concentration evoked greater feeding response than other sugars indicating highest phagostimulation (table 3). Other sugars viz. lactose and maltose did not show such characteristics in case of both the species. Similarly, sucrose at 5% level was found to evoke greater response than maltose, glucose and fructose in case of brown planthopper (Kalode 1971, IRRI 1972). According to Sakai and Sogawa (1976) sucrose significantly stimulated sucking response in this planthopper at concentrations of 15–20% while it was less at concentrations below 10% or above 25%. In case of the smaller brown planthopper *Laodelphax striatellus*, solutions of 5–10% sucrose were found to be the preferred artificial media for oviposition (Mitsubashi and Koyama 1975).

3.3b Response to amino acids: Of the 22 amino acids evaluated, L-alanine, L-glutamine and L-serine were found to be stimulatory in nature, as based on feeding responses of both the species. The responses with these compounds were generally greater in combination with sucrose. Other amino acids, however, showed either deterrent effects or differential feeding reactions (table 4). Asparagine, arginine, leucine and valine strongly enhanced the acceptability of sucrose by brown planthopper while glutamic acid, cysteine and aspartic acid indicated deterrent effects (Kalode 1971). Aspartic acid, alanine, glutamic acid, asparagine and valine were reported to be stimulatory at concentrations ranging from 0.1–1% (Sogawa 1973). Lower asparagine contents in the resistant rice variety Mudgo has been attributed to be one of the factors responsible for resistance (Sogawa and Pathak 1970; Sogawa 1973). Shigematsu *et al* (1982) reported that asparagine stimulated brown planthopper feeding while β -sitosterol inhibited sucking of the sap. It is significant to note from the present investigation that serine and alanine, both phagostimulants were recorded in considerably higher concentrations in the susceptible variety TN 1 than in resistant varieties and least in the weed *L. hexandra*.

3.3c Response to amino acid derivatives: Bioassay of 48 amino acid derivatives showed that most of them, except β -alanine, L-amino-n-butyric acid and Y-amino-n-butyric acid were deterrent in nature. DL-ethionine, 5-hydroxytryptophan, indole-3-

Table 3. Effect of different sugars on the feeding behaviour of *N. virescens* and *N. nigropictus*.

Compound	Relative feeding response			
	<i>N. virescens</i>		<i>N. nigropictus</i>	
	1%	5%	1%	5%
Fructose	+22	+35	+99	+42
Glucose	+33	+77	+37	+57
Lactose	-27	-16	+28	-10
Maltose	-22	-44	+44	+21
Sucrose	+66	+100	+71	+100

–, Negative response. +, Positive response.

Table 4. Effect of different amino acids on the feeding behaviour of *N. virescens* and *N. nigropictus*.

Amino acids	Relative feeding response			
	With distilled water		With 5% sucrose	
	<i>N. virescens</i>	<i>N. nigropictus</i>	<i>N. virescens</i>	<i>N. nigropictus</i>
L-Alanine	+ 50	+44	+72	+46
L-Arginine	-17	+25	+13	+35
L-Asparagine	-14	+ 2	+45	+32
L-Aspartic acid	-12	-16	+25	+33
L-Cysteine	+55	-10	+ 4	+23
L-Cystine	- 1	- 1	+15	+17
L-Glutamine	+38	+30	+50	+70
L-Glutamic acid	-18	-27	+24	+20
Glycine	+13	+34	+52	+44
L-Histidine	+ 1	-11	+50	+56
L-Isoleucine	-43	-12	+ 1	+46
L-Leucine	-50	-22	+15	+12
L-Lysine	-31	-27	- 2	-16
L-Methionine	-37	-20	- 4	- 1
L-Proline	-50	-17	-10	-15
L-Phenylalanine	-37	-28	- 4	- 6
L-Serine	+25	+42	+65	+57
L-Threonine	-50	-14	- 7	+ 4
L-Tryptophan	-43	-24	- 5	+ 2
L-Tyrosine	-18	-32	+ 1	- 3
L-Valine	-21	- 4	-10	+ 6

-, Negative response, +, Positive response.

Table 5. Effect of selected amino acid derivatives on the feeding behaviour of *N. virescens* and *N. nigropictus*.

Amino acid derivatives	Relative feeding response			
	With distilled water		With 5% sucrose	
	<i>N. virescens</i>	<i>N. nigropictus</i>	<i>N. virescens</i>	<i>N. nigropictus</i>
DL-Arenaline	-156	- 94	-125	-104
β -Alanine	+ 13	+ 8	+ 42	+ 36
L-Amino-n-butyric acid	+ 12	+ 16	+ 28	+ 20
γ -Amino-n-butyric acid	+ 4	+ 10	+ 16	+ 24
DNP-L-asparagine	-106	-161	- 65	- 77
DNP-L-glutamine	-156	- 80	-103	- 97
DNP-L-tryptophan	-158	-157	-202	- 40
O-DNP-L-tyrosine	-197	- 90	-155	- 70
DL-Ethionine	-247	-177	-182	-167
Gnanidinobutyric acid	-211	-192	-200	-152
Histamine	-190	- 90	-161	-240
5-Hydroxytryptophan	-214	-172	-208	-160
Indole-3-aldehyde	-226	-182	-232	-182
Indole-3-butyric acid	-188	-155	-211	-165
Indole-3-propionic acid	-273	-210	-232	-152
DL-Methyltryptophan	-244	-120	- 76	- 52
DL-Methionine sulfone	-263	-172	- 36	- 50
DL-Noradrenaline	-186	-152	-126	-122

-, Negative response. +, Positive response.

Table 6. Effect of organic acids and phenolic compounds on the feeding behaviour of *N. virescens* and *N. nigropictus*.

Compound	Relative feeding response			
	With distilled water		With 5% sucrose	
	<i>N. virescens</i>	<i>N. nigropictus</i>	<i>N. virescens</i>	<i>N. nigropictus</i>
Organic acids				
Citric acid	-166	-160	-111	-115
Fumaric acid	-72	-80	-51	-75
Phenolic compounds				
Benzoic acid	-111	-125	-108	-104
Caffeic acid	-183	-105	-112	-105
Catechol	-177	-140	-221	-170
Cinnamic acid	-172	-95	-165	-140
Coumaric acid	-155	-165	-183	-150
Hydroquinone	-161	-120	-188	-145
Hydrobenzoic acid	-188	-105	-167	-155
Phloroglucinol	-105	-80	-161	-220
Protocatechuic acid	-138	-145	-172	-184
Pyrogallol	-150	-180	-105	-100
Quinon	-133	-90	-122	-110
Salysilic acid	-72	-70	-134	-74
Sulfanilic acid	-94	-85	-130	-140
Vanillic acid	-138	-90	-117	-105
Vanillin	-111	-180	-105	-135

—, Negative response.

aldehyde, indolepropionic acid, DL-methyltryptophan and DL-methionine sulfone indicated greater inhibitory reactions, particularly against *N. virescens* (table 5). Rest of the amino acid derivatives indicated intermediate inhibitory reactions. Few of the decarboxylated derivatives of aromatic amino acids such as phenethylamine, tyramine and hordenine have been reported to be sucking inhibitors of rice brown planthopper at 1000 ppm concentrations (Sogawa 1982).

3.3d Response to organic acids and phenolic compounds: The two organic acids and 15 phenolic compounds bioassayed also behaved as strong feeding inhibitors both for *N. virescens* and *N. nigropictus*, even in combination with 5% sucrose (table 6).

Detailed investigations on varietal resistance in rice against the green leafhoppers had indicated impaired feeding activity as a probable pathway of manifestation of resistance (Viswanathan and Kalode 1987). Present biochemical analysis pointed out the role of certain phagostimulants like the amino acid serine which stimulated feeding response in both the leafhoppers and was found in considerably higher concentration in the susceptible variety TN 1 than in resistant rice varieties. In the context of present biochemical analysis of test varieties and the weed *L. hexandra* which is highly resistant to *N. virescens* but susceptible to *N. nigropictus*, it appears that biochemical nature of resistance operating in test rice varieties may be entirely different than that in the weed against the two species of leafhoppers.

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Polyacrylamide gel electrophoretic analysis of some species specific proteins in six Indian rodent genera (Subfam.: Gerbillinae and Murinae: Fam: Muridae)

M S PRADHAN and A M BHAGWAT*

Zoological Survey of India, Western Regional Station, Shivajinagar, Pune 411 005, India

*Department of Biological Sciences, RJ College, Ghatkopar (West), Bombay 400 086, India

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Abstract. Studies on polyacrylamide gel electrophoresis were undertaken for analysing haemoglobins and eye lens proteins in 6 Indian rodent genera from chaemotaxonomic point of view. The report reveals a distinct segregation of *Rattus* and *Bandicota* from *Mus* and other two murine genera. Revisionary studies of the entire subfamily Murinae for reconsidering the taxonomic placement of *Rattus* and *Bandicota* in the subfamily has been suggested.

Keywords. Polyacrylamide gel electrophoresis; haemoglobins; eye lens proteins; genetic identities; rodent genera; dendograms.

1. Introduction

The Indian rodent genera, particularly the commensal forms like *Rattus*, *Bandicota* and *Mus*, present a most complicated classification. Laborious work of Marshall (1977) for the reconstruction of the taxonomy of *Mus* gives an idea of magnitude of the problem. Raman and Sharma (1977) and Yosida (1973) have reported chromosomal polymorphism in the species of *Rattus*. Selander and Yang (1969) have analysed polymorphism in about 36 proteins including haemoglobin, plasma proteins, specific enzymes etc. and studied genetic heterozygosity in *Mus*. Chromosomal polymorphism is also well known in *Mus* (Marshall 1977). Sharma and Raman (1973), Avirachan *et al* (1971) and Pradhan *et al* (1985) have traced variations in the chromosomes and some of the species specific proteins such as haemoglobins, haptoglobins and transferrins in *Bandicota*. Hence, a necessity is now being felt to compare and study the relationships between the different genera of commensal rodents and confirm their taxonomic placement in the family.

This communication reports the relationship of haemoglobins and eye lens proteins amongst *Rattus*, *Mus*, *Bandicota*, *Tatera*, *Golunda* and *Vandeleuria* (Fam: Muridae) after reviewing the results of polyacrylamide gel electrophoresis (PAGE).

2. Materials and methods

Figure 1 and table 1 show the sampling areas of 6 different rodent genera and the genera-wise samples pooled from the populations. The rodents were collected in the sampling area during extensive surveys carried over a period of 4 years (1982-86). The collection of live specimens from the wild populations was made by using wonder and sherman traps and/or by escavating live burrows. The animals were sacrificed and the haemoglobins in solution were separated from red blood

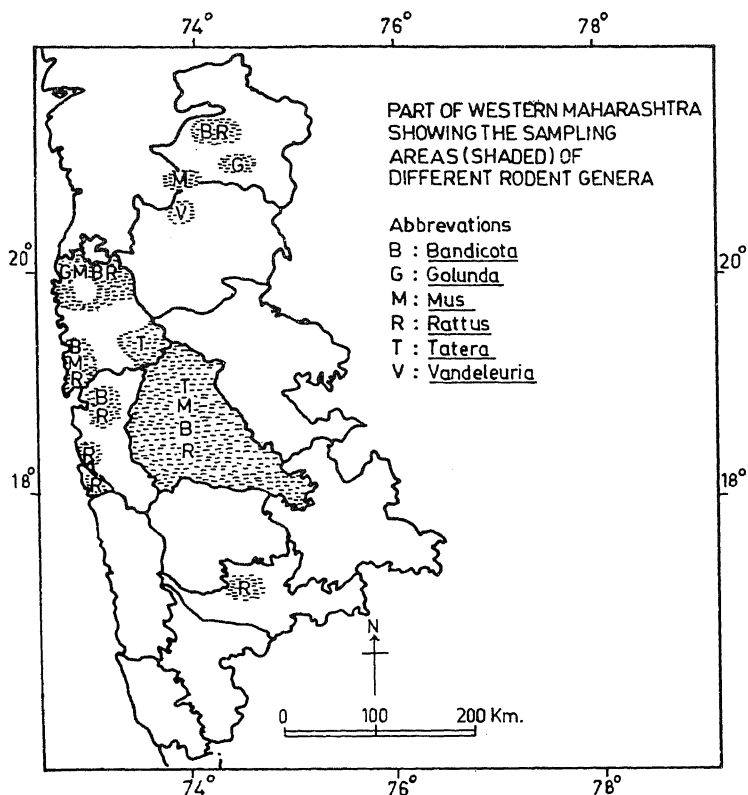


Figure 1. Part of western Maharashtra showing the sampling areas (shaded) of different rodent genera.

Table 1. Sample size of 6 rodent genera pooled from different localities.

Names of genera	No. of species (subspecies studied)	Sampling areas	Sample size
<i>Rattus</i>	2(4)	Dhulia Dist., Thane Dist., Greater Bombay, Pune Dist., Raigad Dist., Sangli Dist.	75
<i>Bandicota</i>	3(3)	Dhulia Dist., Thane Dist., Greater Bombay, Pune Dist., Raigad Dist.	51
<i>Mus</i>	5(8)	Dhulia Dist., Thane Dist., Dang Dist., Greater Bombay, Pune Dist.	25
<i>Golunda</i>	1(2)	Dhulia Dist., Thane Dist.	3
<i>Tatera</i>	1(1)	Thane Dist., Pune Dist.	6
<i>Vandeleuria</i>	1(1)	Nasik Dist.	1

corpuscles as per the methods given by Wright (1974). The methodology used for electrophoretic analysis of both the proteins was mostly based on methods described by Whitaker (1967) and Wright (1974). However, detailed account of the modified methodology adopted is given in the following paragraphs.

Haemoglobins were separated by lysing them in distilled water from isolated red blood corpuscles that were washed to remove plasma contamination and the

mixture was then centrifuged to separate solution containing dissolved haemoglobins and cell debris. The eye lenses were extracted essentially by the method of Smith (1971). Eye lenses of individual specimen were removed from the eye balls immediately after killing the animal. Using a glass homogeniser and 0.012% NaCl solution the soluble lens proteins were extracted. The homogenate was maintained at 0°–4°C for 24 h with intermittent stirring. The extract was then centrifuged at 5000 rpm for 20 min in a refrigerated centrifuge (Temp. 4°C ± 1°C) to obtain a clear extract containing soluble eye lens proteins. The protein extracts, after treatment of sodium dodecyl sulphate (SDS), in the presence of mercaptoethanol were used for SDS-PAGE.

Polyacrylamide gel (7.5%), stacked at pH 8.3 and running at pH 9.5, was used for the separation of haemoglobins and eye lens proteins. The gel solutions were cast in neutral glass tubes (size: 0.6 × 10 cm). Ammonium per sulphate (0.14%) freshly prepared and added to gel solution before casting in tubes served as an additional catalyst. Each glass tube contained 2 ml of running gel and 0.1 ml of 5% spacer gel. The buffer with 8.3 pH used in the system contained 6 g of Tris and 28.8 g of glycine dissolved in one litre of distilled water. Before filling the chambers of electrophoretic assembly the buffer solution was diluted 10 times with distilled water. Haemoglobin solutions/eye lens extracts were loaded on the gel columns in each tube after mixing the sample solution with marker, bromophenol blue. The electrophoretic run was carried out at 4°C ± 1°C at a constant voltage (200 V) and current (3–4 mA) per tube. The separation was terminated after 3 h, the time by which marker migrated to the other end of the tube. After completion of the electrophoretic runs, the gel columns were stained by benzedene coupled reaction (Ornstein 1967) for identifying the haemoglobin bands, while the lens proteins were stained with 0.1% methanolic Coomassie brilliant blue R-250 (Gordon 1980). Consolidated protein profiles were prepared by analysing each sample in several replicates and averaging the electrophoretic mobilities with reference to the marker (R_m values) for individual specimens. The final R_m values obtained for the individuals were clubbed together to obtain characteristic profiles for the 6 genera under investigation.

The identification of the individual specimen was done and confirmed by one of the authors as per Ellerman (1961), Marshall (1977) and Roonwal and Agarwal (1962). The voucher specimens have been registered with Western Regional Station, Zoological Survey of India, Pune.

3. Results and discussion

Figure 2 shows a diagrammatic representation of the consolidated electrophoretic patterns of the selected rodent genera. It depicts the patterns for haemoglobins (6 genera) and eye lens proteins (3 genera). The polymorphism at the gene locii regulating the synthesis of both these proteins in *Rattus* and *Bandicota* have been reported elsewhere (Pradhan *et al* 1989). In spite of the polymorphism at the gene locii for haemoglobin, a single band haemoglobin pattern representing homozygosity was observed in individuals of atleast 4 genera. However, Pradhan *et al* (1989) have reported minimum expression of 3 band patterns for eye lens proteins in *Bandicota*. Three basic band patterns of eye lens proteins were also recorded from some of the *Rattus* and *Mus* samples pooled for the current studies.

The haemoglobin profiles (figure 2) of *Rattus* and *Bandicota* show bands

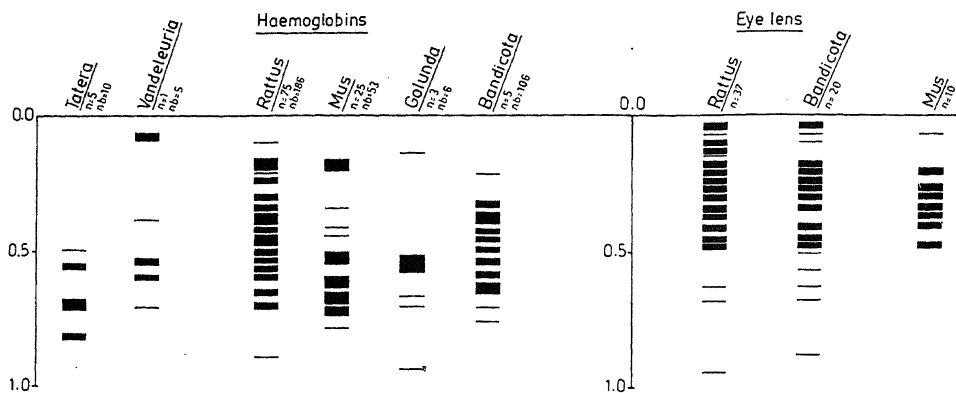


Figure 2. Diagrammatic representation of the electrophoretic patterns of the species specific proteins in the 6 rodent genera.

distributed over the entire R_m range, whereas the profile for *Mus* haemoglobins has a restricted distribution of bands (either mostly below 0.5 or around 0.1). The polyacrylamide gel electrophoretic runs of individual *Mus* specimens were very characteristic and distinctly different. With the exception of members from *Mus booduga*, *Mus saxicola* and *Mus musculus urbanus*, large number of *Mus musculus* samples showed total absence of haemoglobin bands in the R_m range between 0.1–0.5. The lighter fractions were seen either singularly or in combination with the heavier fractions depicting characteristic *Mus* patterns. Similar observations were made in the specimens pooled from *Tatera*, *Golunda* and *Vandeleuria* populations also.

Though extensive variations are obvious in the eye lens protein profiles of *Rattus* and *Bandicota*, variations appear to be limited in *Mus*. As stated earlier, the basic 3-band pattern in an individual, was seen in all the 3 genera.

Thus, a careful comparison of both the profiles in all the 6 genera suggests the presence of two separate groups. *Rattus* and *Bandicota* fall in one group with almost identical pattern of variations, while *Mus*, *Tatera*, *Golunda* and *Vandeleuria* show significant resemblances amongst each other in the haemoglobin profiles. *Mus* also exhibits a distinctly different eye lens protein profile when compared to *Rattus* and *Bandicota*.

For better insight of the relationship of these rodents, genetic identities and distances (Nei 1972) in both the proteins were calculated and compared. Table 2 shows that *Tatera*, *Mus*, *Golunda* and *Vandeleuria* fall in one group with absolute genetical identity for haemoglobin loci. It further shows that *Rattus* is closely related to *Bandicota*, the genetic identity being 0.90. Similarly, loci for *Mus* eye lens proteins show maximum genetic distance from those for *Rattus* and *Bandicota*. Based on these values (table 2) the UPGMA method of Sneath and Sokal (1973) was applied to construct a dendrogram for the 6 rodent genera (figure 3). It portrays a clear separation of *Rattus* and *Bandicota* from *Mus* and its associates forming independent groups. The level of similarity for *Mus*, *Tatera*, *Golunda* and *Vandeleuria* was absolute for the haemoglobin loci. Moreover *Rattus*, *Bandicota* and *Mus* are not at all closely related when levels of similarities for eye lens proteins were considered, *Mus* being placed further ($I=0.44$) from *Rattus* and *Bandicota*.

Table 2. Estimates of genetic identity (above diagonal) and genetic distance (below diagonal) among the members of 6 genera of Murinae (Fam: Muridae, order: Rodentia) based on haemoglobins and eye lens proteins analysis on PAGE.

Haemoglobins			Eye lens proteins		
<i>Tatera</i> , <i>Vandeleuria</i> , <i>Golunda</i> , <i>Mus</i>	<i>Rattus</i>	<i>Bandicota</i>	<i>Rattus</i>	<i>Bandicota</i>	<i>Mus</i>
—	0.62	0.62	—	0.48	0.41
0.49	—	0.9	0.47	—	0.47
0.49	0.11	—	0.91	0.76	—

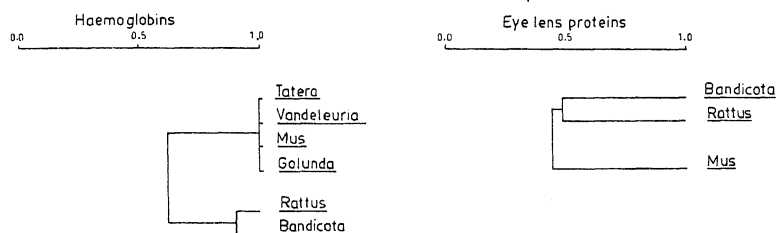


Figure 3. Dendograms showing the relationships of 6 rodent genera generated according to UPGMA method.

The above results would raise some doubts and speculations. However, a second thought will help to resolve some problems in the taxonomic placements of these genera in the family. On the basis of Goodman's (1976) data on phylogeny of various organisms, Fergusson (1980) has doubted the placement of *M. musculus* and *Rattus norvegicus* in the same subfamily, Murinae. Jacobs (1978) and Jacobs and Pilbeam (1980) on the basis of immunological data and information derived from analysis of amino acid sequences do not feel that *Rattus* and *Mus* are phylogenetically close. Musser and Newcomb (1983), after examining the native murid material from Malaya and Sumatra have reached a conclusion that the relationship of *Rattus* with other genera is obscure. The present observations also clearly show that a revision of the taxonomic placements of some of the murine rodent genera has, now, become essential. Though, Cain (1968) has rightly pointed out that the taxonomic placement of any category should not be based only on the biochemical analysis, it is certain that genus *Mus* is distinctly different in all respects from the remaining murine rodent genera studied for the present work. *Tatera* belongs to an independent subfamily, *Gerbillinae*, though during the present investigations it has shown an absolute genetic identity for haemoglobin loci with the other 3 genera (*Mus*, *Golunda* and *Vandeleuria*). Thus, it is suggested that *Mus*, *Golunda* and *Vandeleuria* be retained in the subfamily, Murinae, and the taxonomic placement of Indian genera *Rattus* and *Bandicota* in the subfamily should be reconsidered. A definite conclusion on the placement of *Rattus* and *Bandicota* in the subfamily Murinae can be drawn only after comparing these two genera with those of murine genera which have not been examined during the present investigations.

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Density and diversity in relation to the distribution of earthworms in Madras

S A ISMAIL*, C RAMAKRISHNAN and M M ANZAR

Department of Zoology, The New College, Madras 600 014, India

*Present Address: Regional College of Education, Mysore 570 006, India

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Abstract. Earthworm density and diversity have been studied in a sandy loam site and a clay loam site, each comprising of 50 locations, at Madras. *Lampito mauritii* is the dominant species in the sandy loam while it is *Octochaetona serrata* in the clay loam. The indices of dominance, diversity and dissimilarity have been estimated and discussed in relation to the distribution of earthworms.

Keywords. *Lampito mauritii*; *Octochaetona serrata*; density; dominance; diversity; dissimilarity.

1. Introduction

Earthworms are among the most widely spread invertebrate animals in the tropical and temperate regions of the world and are found mainly in the soils of forests, woodlands and grasslands which together cover about 80 million square kilometres, or about 54% of land surface of the earth (Whittaker and Likens 1973). Various ecological as well as mechanical parameters influence the size of earthworm populations, their species diversity, dominance and vertical distribution (Edwards 1983; Krishnamoorthy and Ramachandra 1988). *Lampito mauritii* Kinberg, *Drawida modesta* Rao, *Octochaetona pattoni* (Michaelsen), *Octochaetona serrata* (Gates), *Octochaetona thurstoni* (Michaelsen) and *Ramiella pachpaharensis* (Stephenson) have been reported from the soils of Madras (Ismail and Murthy 1985).

The present investigation is a detailed study of the soil system applying principles of quantitative ecology in two sites of Madras each comprising of 50 locations. These sites signify as representations of sandy loam and clay loam soils. Atmospheric temperature, soil temperature, soil moisture, bulk density of soil, pore space, maximum water holding capacity of soil, pH, oxidizable organic matter and plant cover have been investigated; and the population density, dominance, diversity and dissimilarity that exist between the locations in each site with special reference to earthworms have been recorded.

2. Materials and methods

Ecological studies were conducted in two sites of Madras (13°5'N, 80°18'E), categorically being designated as sites A and B. Site A is situated in Ameer Mahal (a stretch of private land in Madras city) where the soil is light brown sandy loam. *Ricinus communis* Linn (Euphorbiaceae), *Acalypha indica* Linn (Euphorbiaceae), *Cleome viscosa* Linn. (Capparidaceae), *Rhynchosia heynei* W and A (Fabaceae), *Boerhaavia diffusa* Linn. (Nyctaginaceae), *Ageratum conyzoides* Linn. (Asteraceae),

Kyllinga sp. (Cyperaceae), *Cyperus* sp. (Cyperaceae) and *Cynodon* sp. (Poaceae) are the floral associates of the community. Of the 50 locations surveyed in site A, locations 44–50 are from a frequently trampled path.

Site B situated in the National Park, Guindy, on the outskirts of the city has brown clay loam. Totally protected and with natural surroundings, this reserve forest has a high density of trees, shrubs and herbs providing shade and litter. *Mullugo* sp. (Aizoaceae), *Oldenlandia* sp. (Cyperaceae) and *Cynodon* sp. (Poaceae) are the floral associates. Of the 50 locations surveyed here, locations 38–50 lie on the path.

Atmospheric temperature (30 cm above soil surface), soil temperature (10 cm below soil surface), soil moisture (Misra 1968), bulk density (dry weight of soil/volume of soil), porosity and water holding capacity (Keen and Raczowski 1921), pH (Hanna 1968) and oxidizable organic matter (Walkley 1947) of the soil samples were estimated in all the 100 locations. In each location 30 cm depth of soil from an area of 0.1 m² was excavated, macrofauna was handsorted and preserved in 5% formaldehyde.

In order to study species composition, the methods used in the study of trophic structure of the ecosystem were adopted. The standing crop per unit area was measured and described. Each collection was individually sorted out, animals belonging to different species were grouped and counted. Using the data so obtained the following indices of species structure in community were assessed:

- (i) Index of dominance (c) (Simpson 1949)

$$c = \sum (ni/N)^2,$$

where ni = number of individuals for each species and N = total number of individuals in that location.

- (ii) Index of similarity (Sorenson 1948)

$$S = \frac{2C}{A+B},$$

where A , B = number of species in samples A and B and C = number of species common to both samples.

Index of dissimilarity = $(1 - S)$.

- (iii) Index of general diversity (\bar{H}) (Shannon and Weaver 1949 cf Odum 1971)

$$\bar{H} = -\sum ni/N \log_e ni/N,$$

where ni = importance value for each species and n = total of importance value.

3. Results

Table 1 provides data for the parameters investigated in the two sites A and B.

Site A is inhabited by the earthworms *D. modesta*, *L. mauritii*, *O. pattoni*, *O. serrata*, *O. thurstoni* and *R. pachpaharensis* in association with insects (*Gryllotalpa* sp. and *Anthia* sp.), myriapods and molluscs (*Ariophanta* sp. and *Vaginulus* sp.). Earthworms constitute 80.18% of the faunal density corresponding to 77.37% of the faunal biomass from this site. Of the earthworm species *L. mauritii* is the most dominant in site A contributing 44% of the biomass followed by

Table 1. Results of the parameters investigated at sites A and B (mean \pm SD).

	Site A	Site B
Atmospheric temperature ($^{\circ}\text{C}$)	26.39 \pm 3.63	28.41 \pm 0.52
Soil temperature ($^{\circ}\text{C}$)	25.20 \pm 0.80	27.14 \pm 0.72
Moisture (%)	15.96 \pm 1.11	17.28 \pm 1.26
Bulk density (g/cm^3)	1.18 \pm 0.05	1.31 \pm 0.07
Porosity of soil (%)	54.98 \pm 6.98	53.23 \pm 8.38
Maximum water holding capacity of soil (%)	47.61 \pm 7.00	55.15 \pm 5.92
pH	6.98 \pm 0.46	7.18 \pm 0.29
Oxidizable organic matter (%)	2.14 \pm 0.47	2.12 \pm 0.49
Plant cover (%)	30.10 \pm 12.43	62.40 \pm 12.00
(g dry wt/0.5 m ²)	5.00 \pm 2.62	10.16 \pm 3.91
Earthworms:		
(nos/m ²)	53.42 \pm 39.22	72.64 \pm 22.67
(g live wt/m ²)	7.32 \pm 5.77	9.77 \pm 4.69
Total macrofauna		
(nos/m ²)	66.67 \pm 44.93	76.86 \pm 22.58
(g live wt/m ²)	9.34 \pm 7.32	10.07 \pm 4.94
Index of dominance (c)	0.50 \pm 0.22	0.64 \pm 0.18
Index of general diversity (H')	0.85 \pm 0.42	0.58 \pm 0.28

Each value is mean of 50 observations.

O. thurstoni (24.62% of the biomass). Arthropods and molluscs contribute to 5.23 and 17.47% of the macrofaunal soil biomass respectively. *O. serrata* which is a dominant form in site B constitutes only 8.2% of the biomass in site A.

Site B is inhabited by the earthworms *D. modesta*, *L. mauritii*, *O. serrata*, *O. thurstoni* and *R. pachpaharensis* in association with coleopteran caterpillars and a scanty representation by arachnids (soil spider) and molluscs. Earthworms constitute 93.2% of the faunal density corresponding to 97.33% of the total biomass at B. Of the earthworm species *O. serrata* is the most dominant organism in site B contributing 72.31% of the total biomass followed by *L. mauritii* (18.96% of the biomass).

Plant cover is extensive in site B (62.4%) than in site A (30.1%), dry weight of the floral community being 101.6 and 50 g/m² respectively.

The average index of dominance at site A is 0.50 indicating that dominance is shared while it is 0.64 at site B indicating dominance by one or two species. It is indeed evident that the dominant species at site B is *O. serrata* (72.31% of the soil biomass). Converse are the results obtained from the index of general diversity; a value of 0.85 for site A indicating greater diversity of faunal population than in site B which has a value of 0.58. This is in conformity with the greater number of species in site A (15) than in site B (9). The index of general diversity forms a mirror image of the index of dominance (figure 1).

4. Discussion

A number of ecological parameters play a vital role in the distribution and abundance of earthworms (Dash and Patra 1977; Lavelle 1984), which may be due

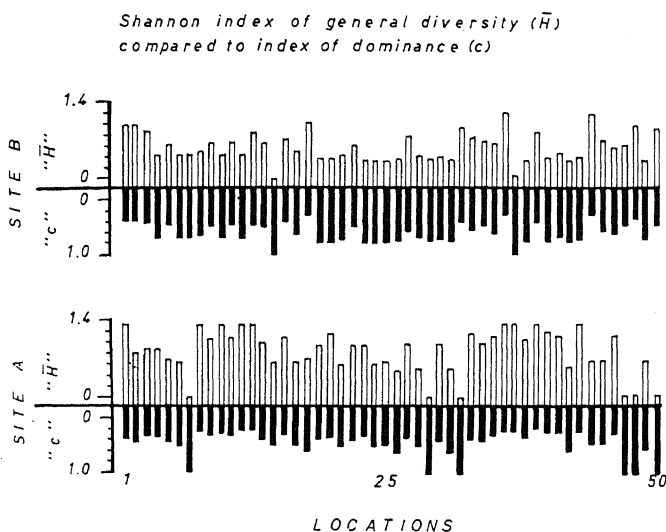


Figure 1. Indices of dominance (■) and general diversity (□) for sites A and B.

to the adaptability of that particular species to the soil sample studied (Lavelle 1983). Temperature, moisture, bulk density of the soil, pore space, water holding capacity and organic matter in soils function as important parameters in the distribution of the earthworms. Though the soil is sandy loam at site A and clay loam at site B, edaphic factors in these two sites are within the preferred range for the distribution of earthworms in Madras (Ismail and Murthy 1985).

Due to the differences observed in the distribution pattern of species in locations of both the sites, it was felt necessary to estimate the index of dissimilarity between locations in each site. The degree of dissimilarity in each site has been represented using a dissimilarity matrix (figure 2). The matrix clearly indicates that the similarity in faunal constitution is not the function of closeness of locations but probably is the result of various other factors as suggested by Dash and Patra (1977) and Lavelle (1983, 1984). There appears to be more similarity between locations of site B while the locations at site A show considerable dissimilarity among themselves. Of particular interest in site A are locations 44–50 which show maximum dissimilarity when compared with any other location of this site. Locations 44–50 in site A represent a path frequented by human and cattle. Earthworm abundance and biomass have been shown to decline as a result of trampling by humans (Chappell *et al* 1971; Aritajat *et al* 1977) and cattle (Briggs 1978). The density of earthworms in these locations due to trampling was just $17.1/\text{m}^2$ (biomass $1.47 \text{ g}/\text{m}^2$) and the total number of macrobiota was $28.6/\text{m}^2$ (biomass $1.63 \text{ g}/\text{m}^2$).

Observations reveal that the faunal associates are reduced to about 20 in the region of the path in site A but trampling does not seem to have such an effect in site B where locations 38–50 are also representations of path. At site B the floral associates are reduced by 20%, i.e. 80% of the plant cover of that present at the other locations exists. Results on the effect of trampling conform to the findings of Pearce (1984) and it could be added that the results of trampling have a more pronounced effect on sandy loams altering their faunal structure.

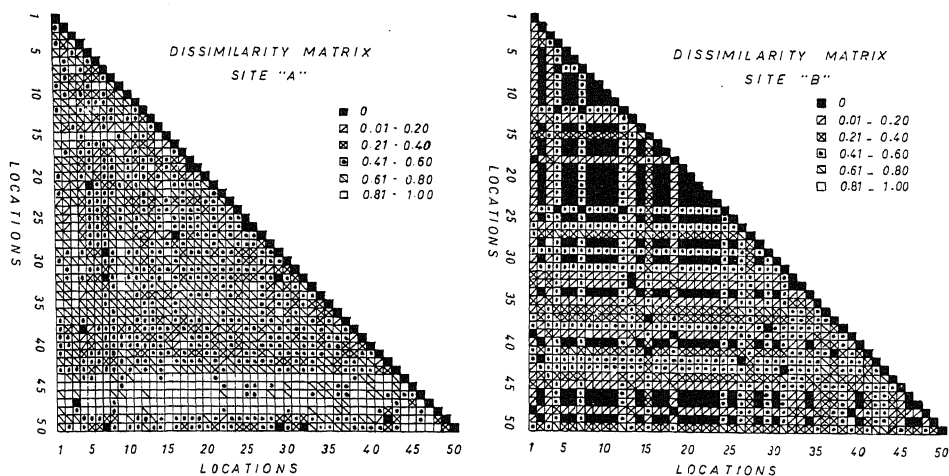


Figure 2. The dissimilarity matrix for sites A and B.

The macrobiota of site B is chiefly represented by the earthworms, the dominant species being *O. serrata*. Soil here being a clay loam, *O. serrata* successfully inhabits this site making use of its powerful buccopharyngeal musculature for eating through the soil. The dissimilarity matrix (figure 2) clearly indicates that the clay loam soil does not favour diversity of species and the degree of dominance is generally concentrated to one or two species. Such dissimilarity matrices (figure 2) would also be useful to the students of field biology in deferring from generalizing the distribution of organisms with a few samples investigated. Each site therefore possesses a region of different density, dominance, diversity and dissimilarity in its organisation.

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Birds of the man-made ecosystems: the plantations

R J RANJIT DANIELS, MALATI HEGDE and MADHAV GADGIL

Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India

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Abstract. One-hectare plots were sampled for bird species diversity in the Uttara Kannada district. These plots represented well-preserved evergreen/semievergreen forests, secondary/moist deciduous forests showing different levels of degradation by man and plantations of teak, eucalypts and betelnut. It was found that the betelnut plantation and the evergreen/semievergreen forests had the least bird species diversity of $H' = 2.58$ and 2.61 respectively. The eucalypt and teak plantations had $H' = 2.69$ and 2.92 respectively. In the secondary/moist deciduous forests it ranged from 2.80 – 3.39 . Despite the apparent increase in diversity in the man-modified vegetation types, it was found that there was a gradual displacement of the bird species composition from what was typical to the evergreen forests to those of more urban and scrubby habitats in these man-modified vegetation types. This was particularly so in the eucalypt plantation.

Keywords. Western ghats; birds; plantations.

1. Introduction

Converting natural forests into plantations has been a practice for at least two centuries in south India. The western ghats have been quite exploited this way. Large patches of rubber (*Hevea brasiliensis*), tea (*Camellia sinensis*), coffee (*Coffea* sp), cardamom (*Elattaria cardamomi*), betelnut (*Areca catechu*), teak (*Tectona grandis*), eucalypts (*Eucalyptus* spp), etc. have become permanent elements of the western ghats landscape today. This transformation of one vegetation type into another could have made the region less fit for one species making it more suitable for another as Schemske and Brokaw (1981) suggest of periodic disturbances in tropical forests. They state that, periodic disturbances can influence the abundance of a particular species of bird and thereby the community diversity.

The first impression that any of these plantations gives an observer is that the birds present in them are a result of influence by the neighbouring natural vegetation. This is particularly exaggerated in plantations that are fairly old and in the middle of natural forests where often many of the forest species like trogons, racket-tailed drongos, bronzed drongos, minivets and woodpeckers forage as mixed flocks (R J Ranjit Daniels, unpublished results). Some species of birds freely use these plantations for foraging and even breeding. The little scalybellied green woodpecker (*Picus myrmecophoneus*) has been reported as being 'partial' to the rubber plantations in south India (Ali and Ripley 1983). The little spiderhunter (*Arachnothera longirostris*) is almost always met with in betelnut plantations and banana plantations if they are near humid natural forests (R J Ranjit Daniels, unpublished results).

Controversial ideas such as monocultures leading to reducing species diversity, local extinctions of species, etc. are currently in vogue. Besides these controversial ideas often reaching the local people through various forms of news media, active voluntary movements such as the Appiko in Uttara Kannada have organised anti-

eucalypt rallies many a times. Scientific investigations on these matters therefore seemed important to us.

While it is difficult to quantify the extent of influence of any kind caused by this displacement of vegetation on the bird communities of the western ghats, it is relatively simpler to get answers to questions like what level of bird species diversity can a plantation support and how similar is the bird community to the adjacent natural forests? Our study tries to answer these two basic questions.

2. Study area

The Uttara Kannada district (13 55'–15 32'N; 74 5'–75 5'E) Karnataka, India (in which the study was carried out) holds approximately 175 km of the western ghats within its political limits. The 250 cm of annual rainfall is chiefly distributed over 5 months, June–October. In altitude, the district ranges from sea level to about 1000 m above MSL. The study sites however were distributed over altitudes of less than 50 m to about 600 m. The plantations in this area are mainly betelnut, teak and eucalypts. The natural forests are of the evergreen/semievergreen type along the slopes and secondary/moist deciduous towards the more even plateau east of the ridge. Together they cover about 80% of the district's total area of 10,200 km². These forests have been legally classified into reserved forests (maintained by the state), minor forests (nearer human habitations and left for their use) and 'Soppina bettas'. The 'Soppina bettas' are like the minor forests but maintained and used by the betelnut growing farmers to meet their leaf-manure requirement, thus they often have better tree growth than the minor forests. The trees on these are periodically cut to manure their betelnut plantations. The best forests are generally the reserve forests. Figure 1 shows the location of the study plots in which birds were sampled. Table 1 gives the localities with their corresponding vegetation types. The legal status of these forests have determined the condition of the vegetation and not the vegetation type itself. The betelnut plantations are distributed as patches over about 50% of the district. They are old, well-watered. The canopy is closed and continuous, often merging with adjacent natural forests. A multi-tiered system of cropping including banana, pepper, cardamom, cocoa, etc. has made this plantation structurally similar to the natural evergreen/semievergreen forests in the district. This land use practice has been the tradition for more than 200 years among the 'Havik brahmins'. The teak and eucalypt plantations which are distributed over 29 and 10% of the district respectively, are more open, without the structural complexity as in the betelnut plantations, drier (especially the eucalypt), and much younger. However, they often have dense undergrowth of thorny, secondary species of plants (Daniels 1989).

3. Methods

The Uttara Kannada district was broadly divided into two vegetation zones which is primarily based on the annual rainfall and the corresponding vegetation types (Pascal 1982, 1984, 1986). Thus we have the evergreen/semievergreen zone and the drier secondary/moist deciduous zone (figure 1).

Three plots, each one hectare (100 × 100 m) in size were laid per vegetation type.

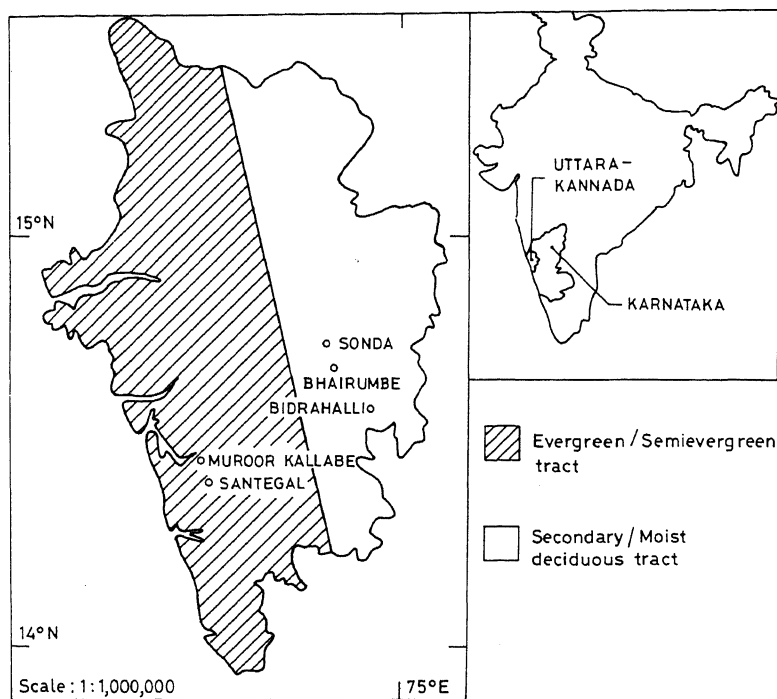


Figure 1. Map of Uttara Kannada showing the broad vegetation regimes and the location of the study plots.

The 4 corners of these plots were permanently marked. Birds were sampled while walking over equally spaced transects (100 m long; 20 m intervals). This procedure was followed only to ensure that equal effort went into sampling all the study plots. All birds seen or heard within the plots and those flying at about 30 m height above ground over the plots were also counted. This height was fixed considering the average height of the canopy in all our study plots to be less than 30 m. All sampling was done between 08:00 and 10:00 h and between December and March when the birds are the most noisy and conspicuous. The 3 plots in each vegetation type were sampled on 3 consecutive days between December 1983 and March 1984 in the natural vegetation and then from January 1985 to March 1985 in the plantations. Since there were 3 plots to represent each vegetation type in the different localities, data from only one sample from each plot is used in this analysis. For calculating overall averages of bird species diversity corresponding to the two zones, more samples from one-hectare plots were used. Thus we have 37 samples from 21 one-hectare plots in the secondary/moist deciduous zone and 31 from 15 one-hectare plots in the evergreen/semievergreen zone. Some plots were sampled during other seasons also. The variation between samples from the same plot has been discussed elsewhere (Daniels 1989).

The species diversity of birds in each of the plant communities was estimated using the Shannon-Weaver diversity index,

$$H' = - \sum p_i \ln p_i$$

where, p_i = the proportion of the i th species in the sample (Pielou 1975).

Table 1. The different vegetation types studied; their location and bird species diversity.

Locality	Latitude (N)	Longitude (E)	Altitude (m > MSL)	Vegetation type/ plant community	No. of bird species	No. of individuals	$-\sum p_i \ln p_i$
Santagal	14°23'	74°30'	375	Evergreen/semi-evergreen (reserve forest)	22	92	2.61
Bidrahalli	14°34'	74°55'	560	Secondary/moist deciduous (reserve forest)	22	93	2.85
Sonda	14°45'	74°49'	520	Secondary/moist deciduous (reserve forest)	26	130	2.80
Bhairumbe	14°41'	74°50'	520	Secondary/moist deciduous (minor forest)	42	258	3.30
Bhairumbe	14°41'	74°50'	520	Secondary/moist deciduous (Soppina betta)	41	154	3.39
Sonda	14°45'	74°49'	520	Teak (plantation)	27	95	2.92
Bidrahalli	14°34'	74°55'	560	Eucalyptus (plantation)	21	80	2.69
Muroor/Kallabe	14°27'	74°29'	50	Betelnut (plantation)	17	38	2.58

To check for similarity in bird species composition and abundance between the vegetation types, the Morisita–Horn similarity index,

$$C\lambda = 2\sum n(1, i) \times n(2, i) / (\lambda(1) + \lambda(2)) N(1) \times N(2),$$

where, $\lambda(j) = \sum n^2(j, i) / N^2(j)$, $n(j, i)$ is the number of individuals of species i in sample j and $N(j)$ is the number of individuals in sample j , was used (Wolda 1981).

For calculating diversity and similarity indices, data from all the 3 hectares of each vegetation type were pooled for each locality.

4. Results and discussion

4.1 Bird species diversity

The answer to the first question viz. what levels of bird species diversity a plantation can support is apparent in table 1. On the whole, the secondary/moist deciduous vegetation types have a higher bird species diversity than the evergreen forests. If averaged, the samples of birds from the moist deciduous zone have a higher diversity ($H' = 2.8$) than those from the evergreen/semievergreen tract ($H' = 2.56$). This difference is significant ($Z = 2.057$; $n(1) = 37$; $n(2) = 31$; $P < 0.05$). The secondary/moist deciduous zone is more heterogeneous spatially with a lot of second growth and openings (Daniels 1989). Such habitats are known to be more productive (Schemske and Brokaw 1981). Also apart from being secondary, these seem to be seral. This may be a further reason for the increased level of bird species diversity (Connell 1978). Except the *Eucalyptus* plantation ($H' = 2.69$), the other vegetation types in the secondary/moist deciduous zone have diversities equal to or greater than the corresponding average viz. $H' = 2.8$ (table 1). It appears from our results that there is an increase in bird species diversity when forests are disturbed (Daniels 1989). Connell (1978) relates high levels of diversity to disturbed conditions and lower levels to stability within the tropics. Among the 3 plantations viz. betelnut, teak and *Eucalyptus*, the first two have diversity of bird species close to their respective vegetation zones. The teak and betelnut plantations which are often allowed to mature for at least 50 years, probably favour a community of birds to colonise, and even stabilize at levels of diversities close to what is appropriate to their respective vegetation zones (Daniels 1989). Frequent disturbances can prevent bird communities from establishing in any vegetation type. 'Establishing' need not necessarily mean 'to breed'. A species finding sufficient food in any habitat eventually becomes dependent on it. For example, a lot of forest birds such as racket-tailed, bronzed and haircrested drongos, lorikeets, blossom-headed parakeets, whiteheaded and hill mynas visit eucalypt trees for nectar. Nectar is an important source of sugar for a lot of otherwise insectivorous birds (Ali and Ripley 1983). The lower species diversity in the eucalypt plantation may be attributed to the fact that the plantation studied was young and cut every 5 years for pulpwood. It may also be true that a plantation of plants as exotic as the *Eucalyptus* may never be able to support a bird community with a level of species diversity equal to the natural forests in its neighbourhood.

4.2 Similarity in bird species composition between the vegetation types studied

Figure 2 answers the second question viz. how similar are the species of birds in the

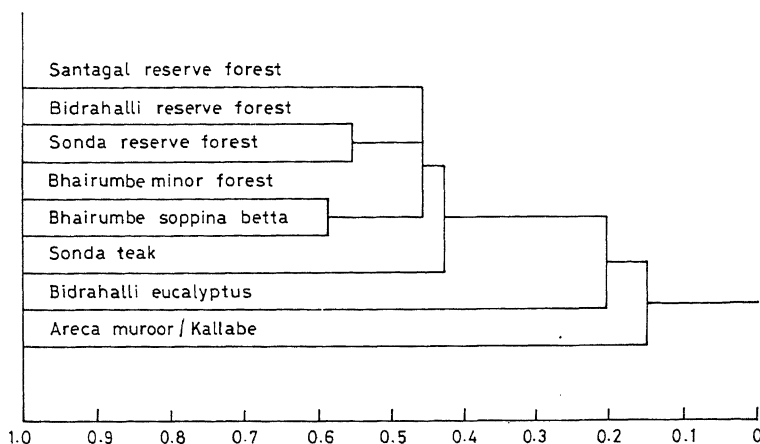


Figure 2. Dendrogram of similarity in the bird communities between the 8 vegetation types studied.

different vegetation types? The 3 better preserved forests viz. Santagal, Bidrahalli and Sonda, in order of increasing disturbance, group together. Similarly, the 3 plantations. The minor forest and the 'Soppina betta' of Bhairumbe not only are the most similar, but also behave as transitions between the well-preserved natural forests and the man-made plantations. They are the most disturbed forests though not fully transformed as the plantations. They share species like *Hirundo daurica*, *Corvus macrorhynchos*, *Lanius cristatus*, *Oriolus oriolus*, etc. which are common urban birds, with the plantations and also birds like the *Irena puella* and *Nectarinia minima* with the better preserved forests (see appendix 1).

Tables 2-4 show how the bird species in each vegetation type have been characterized based on their food preference, habitat preference and geographic distribution over the country (see also appendix 1). This analysis has explicitly shown the differences in the species composition in the 8 vegetation types studied and how each compares with the overall avifauna of Uttara Kannada district.

As can be seen there is an apparent trend of change in the species composition from the natural forests to the completely man-made plantations. The birds preferring the humid evergreen forests are highly represented in Santagal (77.27%; table 2), the best forest among the 8 studied, and poorly represented in the *Eucalyptus* plantation (4.76%). On the contrary, birds of drier thicket habitats such as *Prinia hodgsoni* has invaded the eucalypt plantation. There is also a gradual increase in the proportion of generalist bird species (those which use more than one of the described habitat types) represented in the 8 vegetation types studied, starting with Santagal (22.73%) to the *Eucalyptus* plantation (76.19%).

Those species of birds which are predominantly frugivorous are found in a greater proportion in the evergreen/semievergreen forests of Santagal (45.45%) and the least in the betelnut plantation (5.88%). The *Eucalyptus* plantation has 14.29% (table 3). The reverse is true of the insectivorous birds. The betelnut and the *Eucalyptus* plantations have 76.47 and 71.43% respectively.

Table 4 shows that the endemic forms (species and subspecies) of birds of the western ghats are more in Santagal (45.45%) and the least in the *Eucalyptus*

Appendix 1. The complete list of birds identified during sampling in the 8 vegetation types with details of their geographic distribution (countrywide), food and habitat preferences and occurrence in the different vegetation types. For interpretation of codes see tables 1-4.

Sl. No. ^a	Species	Geographic distribution	Habitat preference	Food	Occurrence in study area
42	<i>Ardeola grayii</i>	bcdefgh	c	a	3
44	<i>Bubulcus ibis</i>	bcdefgh	g	c	7
496	<i>Treron pompadora</i>	a	a	b	256
504	<i>Treron phoenicoptera</i>	b	b	b	45
511	<i>Ducula badia</i>	a	a	b	1
537	<i>Streptopelia chinensis</i>	bcdeg	g	e	4
542	<i>Chalcophaps indicus</i>	bcefg	a	e	5
558	<i>Psittacula cyanocephala</i>	b	g	e	234567
566	<i>Loriculus vernalis</i>	bcdeh	g	e	12345678
582	<i>Cacomantis sonneratii</i>	bde	b	c	5
602	<i>Centropus sinensis</i>	b	g	c	4
692	<i>Chaetura sylvatica</i>	efg	a	c	1245
694	<i>Apus melba</i>	bcdefg	g	c	6
703	<i>Apus affinis</i>	bcd	f	c	34
707	<i>Cypsiurus parvus</i>	bcd	g	c	4
709	<i>Hemiprocne longipennis</i>	bde	b	c	34
750	<i>Merops orientalis</i>	bcde	g	c	4
768	<i>Tockus griseus</i>	a	a	b	56
775	<i>Anthracoceros coronatus</i>	bd	a	b	6
781	<i>Megalaima zeylanica</i>	a	g	b	124
785	<i>Megalaima viridis</i>	b	g	b	135
790	<i>Megalaima rubricapilla</i>	a	a	b	1
792	<i>Megalaima haemacephala</i>	bcde	g	b	45
804	<i>Micropternus brachyurus</i>	b	b	c	8
820	<i>Dinopium javanense</i>	b	a	c	1
825	<i>Dinopium benghalense</i>	a	g	c	234
852	<i>Picoides nanus</i>	bd	b	c	2456
856	<i>Hemicircus canente</i>	d	a	c	36
862	<i>Chrysocolaptes lucidus</i>	a	a	c	8
927	<i>Hirundo daurica</i>	bcd	g	c	57
922	<i>Hirundo fluvicola</i>	cf	b	c	34
949	<i>Lanius cristatus</i>	bdegh	g	c	457
953	<i>Oriolus oriolus</i>	bcd	g	c	456
959	<i>Oriolus xanthornus</i>	bd	b	c	4
965	<i>Dicrurus leucophaeus</i>	bcd	g	c	2567
967	<i>Dicrurus caerulescens</i>	bcd	b	c	47
971	<i>Dicrurus aeneus</i>	bdeg	a	c	1235
977	<i>Dicrurus paradiseus</i>	b	a	c	346
988	<i>Sturnus malbaricus</i>	a	a	c	4
1016	<i>Gracula religiosa</i>	a	a	b	147
1057	<i>Corvus macrorhynchus</i>	bd	g	f	45678
1065	<i>Hemipus picatus</i>	bd	a	c	45
1068	<i>Tephrodornis gularis</i>	a	a	c	36
1070	<i>Tephrodornis pondicerianus</i>	bde	b	c	4
1072	<i>Coracina novaehollandiae</i>	bd	g	c	457
1079	<i>Coracina melanoptera</i>	bde	g	c	45
1081	<i>Pericrocotus flammeus</i>	b	a	c	123456
1094	<i>Pericrocotus cinnamomeus</i>	a	b	c	3456
1100	<i>Aegithina tiphia</i>	b	b	c	457

Contd.

Appendix 1. Contd.

1104	<i>Chloropsis aurifrons</i>	b	a	c	15
1107	<i>Chloropsis cochinchinensis</i>	bd	g	c	45
1109	<i>Irena puella</i>	begh	a	b	1345
1114	<i>Pycnonotus priocephalus</i>	a	a	b	14
1116	<i>Pycnonotus melanicterus</i>	a	a	b	1236
1120	<i>Pycnonotus jocosus</i>	b	g	b	4578
1128	<i>Pycnonotus cafer</i>	b	g	b	457
1144	<i>Hypsipetes indicus</i>	a	a	b	123
1149	<i>Hypsipetes madagascariensis</i>	b	a	b	13
1224	<i>Rhopocichla atriceps</i>	a	a	c	1
1264	<i>Turdoides striatus</i>	a	g	c	7
1390	<i>Alcippe poiocephala</i>	a	a	c	138
1407	<i>Muscicapa latirostris</i>	bdefh	g	c	25
1411	<i>Muscicapa parva</i>	bcf	g	c	567
1435	<i>Muscicapa pallipes</i>	a	a	c	23
1442	<i>Muscicapa tickelliae</i>	bcde	b	c	58
1460	<i>Terpsiphone paradisi</i>	bdf	g	c	268
1465	<i>Monarcha azurea</i>	bcde	g	c	568
1503	<i>Prinia hodgsoni</i>	bcd	b	c	7
1535	<i>Orthotomus sutorius</i>	bcd	g	c	7
1549	<i>Phragmaticola aedon</i>	bdeh	g	c	3
1556	<i>Acrocephalus dumetorum</i>	bcdef	g	c	137
1602	<i>Phylloscopus trochiloides</i>	bdefgh	g	c	1234567
1661	<i>Copsychus saularis</i>	bcd	g	c	458
1665	<i>Copsychus malabaricus</i>	a	b	c	3
1728	<i>Myiophoneus horsfieldii</i>	a	a	c	18
1734	<i>Zoothera citrina</i>	bd	a	c	8
1811	<i>Parus xanthogenus</i>	a	g	c	5
1838	<i>Sitta frontalis</i>	bdefg	a	c	235
1855	<i>Anthus trivialis</i>	bdf	b	c	6
1874	<i>Motacilla indica</i>	bcdefgh	g	c	57
1884	<i>Motacilla capsica</i>	bcdefgh	g	c	6
1892	<i>Dicaeum agile</i>	bcdef	b	b	4567
1902	<i>Dicaeum concolor</i>	b	a	b	23456
1907	<i>Nectarinia zeylonica</i>	bd	g	c	348
1909	<i>Nectarinia minima</i>	a	a	c	1248
1917	<i>Nectarinia asiatica</i>	bd	g	c	2456
1931	<i>Arachnothera longirostris</i>	beg	a	c	8
1933	<i>Zosterops palpebrosa</i>	bcdefg	g	c	8
1949	<i>Petronia xanthocollis</i>	bcdf	g	e	3456
1968	<i>Lonchura striata</i>	bd	g	e	8

*Serial numbers are those of Ali and Ripley (1983).

plantation (9.52%). It has been found that the birds of the western ghats, especially those of the evergreen/semievergreen forests are amongst the most geographically restricted of Indian birds (Daniels 1989). On these grounds, they have to be assigned a greater conservation value (Nature Conservancy 1983). Contrary to expectation, the 8 vegetation types have failed to show a marked representation of birds of the Assam region in them (table 4). This analysis is important as typicalness of species in a vegetation type makes it more valuable when it comes to conservation assessments (Usher 1986). The birds of the western ghats have a greater affinity to the birds of the northeast (Indo-Chinese region; Ali and Ripley 1983). The more this is expressed in a locality, the more typical the locality is.

Table 2. Habitat preference of the birds found in the 8 vegetation types studied (percentages).

Habitat	Santagal reserve forest	Bidrahalli reserve forest	Sonda reserve forest	Bhairumbe minor forest	Bhairumbe Soppina betta	Sonda teak	Bidrahalli eucalyptus	Muroor/Kallabe betelnut	Uttara Kannada
Humid forest ecosystem	77.27	40.91	53.85	21.43	26.83	37.03	4.76	35.29	21.04
Degraded/open moist forest with grass etc.	—	9.1	11.54	23.81	17.07	14.81	19.05	11.76	12.21
Freshwater marshes	—	—	3.8	—	—	—	—	—	9.35
Marine/estuarine	—	—	—	—	—	—	—	—	3.64
Dry forests with degra- dation/scrub	—	—	—	—	—	—	—	—	12.47
Urban/suburban (close to human habitation)	—	—	3.8	2.38	—	—	—	—	2.08
Those which occur in more than one of the above habitats	22.73	50.00	26.92	52.38	56.10	48.15	76.19	52.94	39.74

Table 3. Food preferences of the birds in the 8 vegetation types studied (percentages).

Food preference	Santagal reserve forest	Bidrahalli reserve forest	Sonda reserve forest	Bhairumbe minor forest	Bhairumbe Soppina betta	Sonda teak	Bidrahalli eucalyptus	Muroor/Kallabe betelnut	Uttara Kannada
Marsh feeders	—	—	3.85	—	—	—	—	—	24.16
Predominantly frugivores	45.45	22.73	23.08	23.81	24.39	22.22	14.29	5.88	5.97
Predominantly insectivores	50.00	59.09	61.54	64.29	63.41	62.96	71.43	76.47	40.78
Carnivores	—	—	—	—	—	—	—	—	12.21
Predominantly herbivores	4.55	13.64	7.69	9.52	9.76	11.11	9.52	11.76	7.79
Omnivores	—	—	3.85	2.38	2.44	3.70	4.76	5.88	9.09

Table 4. Countrywide distribution of the species and subspecies of birds recorded in the 8 vegetation types (percentages)

Geographic distribution	Santagal reserve forest	Bidrahalli reserve forest	Sonda reserve forest	Bhairumbe minor forest	Bhairumbe Soppina betta	Sonda teak	Bidrahalli eucalyptus	Muroor/Kallabe betelnut	Uttara Kannada
Birds restricted to western ghats	45.45	31.82	30.77	16.67	9.76	18.52	9.52	17.65	9.87
Birds found in deccan	50.00	63.64	61.54	78.57	92.68	77.78	90.48	82.35	83.9
Birds found in Indus plain	13.64	13.64	15.38	28.57	29.27	33.33	52.38	35.29	54.03
Birds found in Gangetic plain	22.73	50.00	38.46	57.14	60.98	62.96	71.43	64.71	70.39
Birds found in Assam	27.27	31.82	26.92	28.57	39.02	25.93	33.33	29.41	52.99
Birds found in eastern Himalaya	13.64	22.73	19.23	14.29	24.39	37.03	23.81	11.76	29.87
Birds found in western Himalaya	18.18	18.18	19.23	11.90	19.51	14.81	23.81	11.76	27.53
Birds found in Andaman-Nicobar	13.64	18.18	11.54	9.52	14.63	11.11	23.81	5.88	22.59

Santagal being the least disturbed of the 8 localities studied seems to still retain a community of birds more typical of the western ghats than the other man-modified vegetation types. On the whole, the gradual shift from the specialist-frugivorous birds, the geographically restricted and those typical of the western ghats to the insectivorous generalists with widespread distribution is probably the result of the displacement of the original forests by secondary forests and plantations. Clout and Gaze (1984) report a similar change in the conifer plantations of New Zealand.

5. Conclusions

It appears from this study that the tropical rainforests of the western ghats with their wealth of specialist and endemic species and subspecies of birds are slowly giving way to the more generalist and widespread birds as a result of human interference. That the better preserved evergreen/semievergreen forests of Uttar Kannada and the later introduced plantations fall far apart in terms of similarity in their bird communities and the typicalness of the birds seen in them can be conceived. The plantations however seem to be able to mature/stabilize to support levels of bird species diversity close to that of their vegetation zone. The most drastic shift in the bird community from what is typical to the western ghats to the atypical has taken place in the *Eucalyptus* plantation among the 3 plantation types studied. This study has also shown that by just looking at species diversity of birds we may not be able to visualize the real change in bird communities due to changes in vegetation. Characterizing birds as typical and atypical to any region is a very useful exercise in identifying localities for conservation of birds.

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Aspects of spermiogenesis in *Arrhenothrips ramakrishnae* Hood (Insecta: Thysanoptera)

T N ANANTHAKRISHNAN and A BALU

Entomology Research Institute, Loyola College, Madras 600 034, India

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Abstract. Aspects of spermiogenesis in *Arrhenothrips ramakrishnae* are described based on light microscopic and transmission electron microscopic studies.

Keywords. Spermatids; spermatozoa; axial filament; nebenkern; mitochondrial derivative.

1. Introduction

Aspects relating to the ultrastructure of the sperm cells of thrips and events during spermiogenesis are very much restricted to the works of Bode (1983, 1989). Baccetti *et al* (1969), Baccetti and Afzelius (1976) and Ananthakrishnan (1990). The contributions of Tuzet and Bournier (1951) and Bournier (1956) refer to light microscopic studies on spermiogenesis of *Taeniothrips simplex* (Morison), *Limothrips cerealium* (Haliday) and *Neoheegeria verbasci* (Osborn) and Heming (1976), who have earlier provided useful information in this field. However a detailed study of the succession of events on spermiogenesis as outlined for several species of Lepidoptera has not been available for species of thrips and the present paper attempts to discuss some of the aspects with reference to *Arrhenothrips ramakrishnae*.

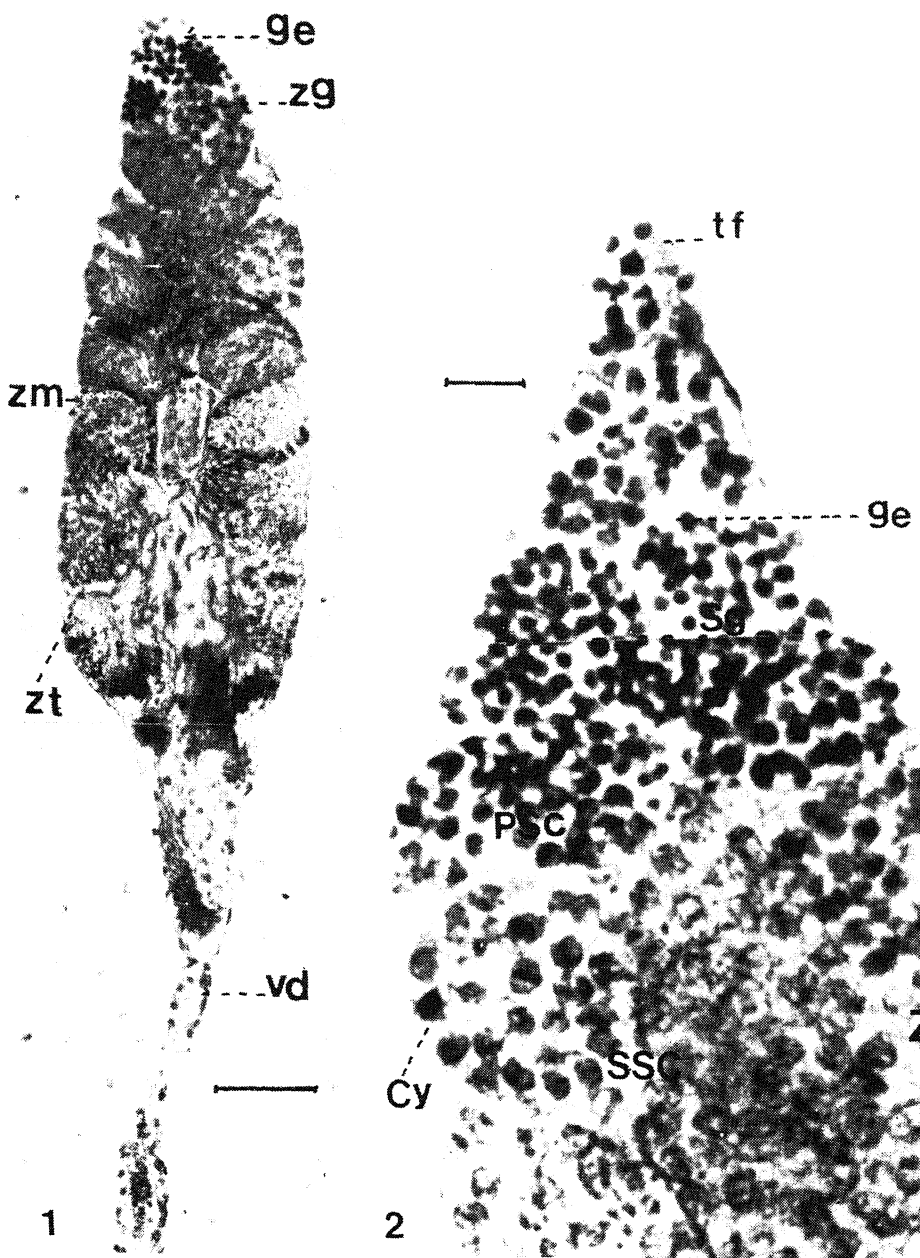
2. Materials and methods

The testes of *A. ramakrishnae* were dissected out in insect ringer solution and were fixed in 2–3% glutaraldehyde + 2–3% paraformaldehyde in phosphate buffer (pH 7.2) (0.1 M). After osmium tetroxide fixation, tissues were rinsed in buffer, keeping them in 1% uranyl acetate overnight at 0.4°C and then dehydrated. Eventually the material was embedded in an Epon-Araldite mixture and polymerized at 60°C and ultra thin sections cut with an ultratome and examined in a transmission electron microscope.

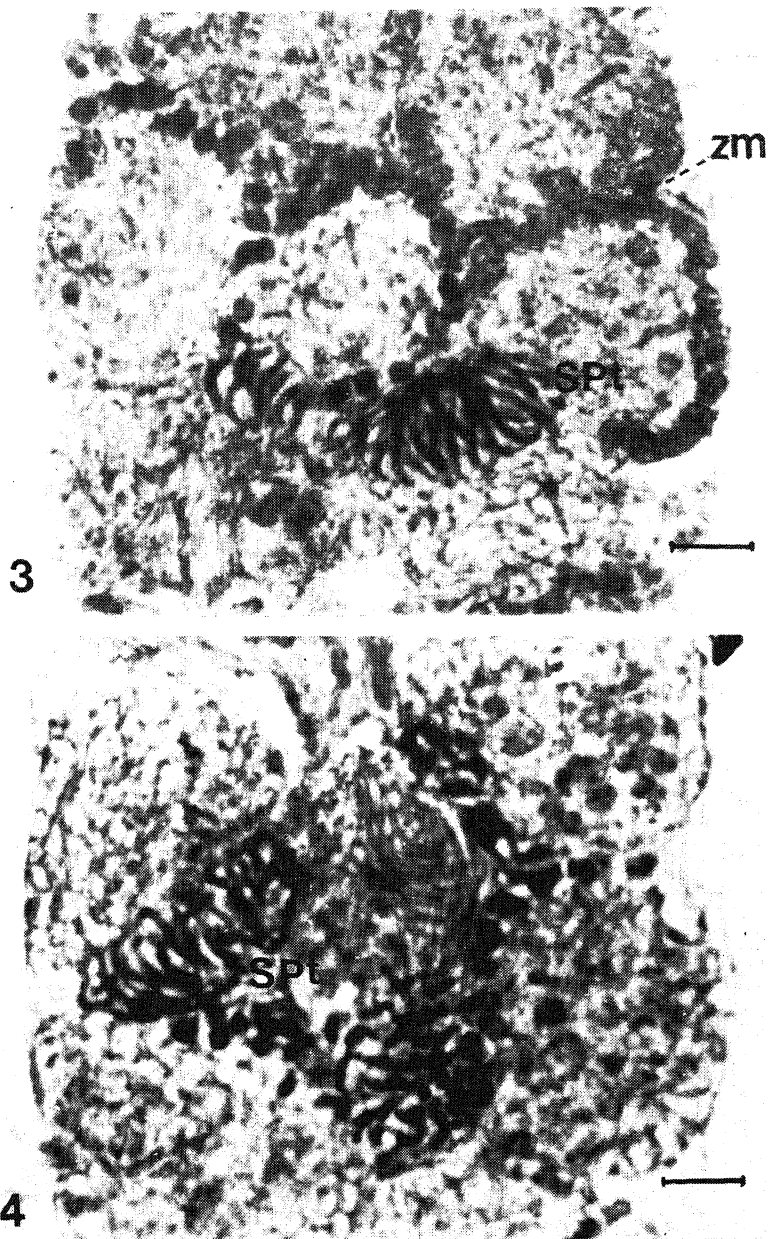
3. Results

Light microscopic studies reveal the numerous cysts packed with sperm cells in different stages of maturation or development distributed from the proximal to the distal end of the follicle. The germarium which is a conical part of the follicle consists of groups of darkly stained, small spermatogonial (Sg) cells, followed by the zone of growth (Zg) containing the primary (PSC) and secondary spermatocytes (SSC) in much enlarged cysts (figure 2). The Sg cells appear as masses in the germarium packed inside the cysts are arranged in 3 rows along the width of the

follicle in Zg. The small Sg cells increase in size with various meiotic divisions and are spread loosely within the cysts. Such a type of definite arrangement of cysts in 3 rows continue to be maintained till the end of zone of maturation (Zm) (figure 4). In Zm the developed SSC inside the cysts move spirally and on



Figures 1 and 2. 1. LS of a testis showing the different stages of the development of sperms (bar = 15 μ m). 2. Enlarged view of the germarium showing the PSC and SSC inside the cysts (bar = 10 μ m).



Figures 3 and 4. Formation of spermatids in the Zm (bar = 10 μ m).

towards the centre of the follicle along the periphery of each cyst. Such cells become darkened with well developed nuclei (figure 4). These cells in the periphery of the cysts group together in the centre of the follicle and are differentiated into numerous spermatids (figure 5). By this time the cysts of this zone completely lose their intercystic boundaries. Later in the zone of transformation (Zt) the differentiated spermatids are transformed into spermatozoa (Spz) (figure 3).

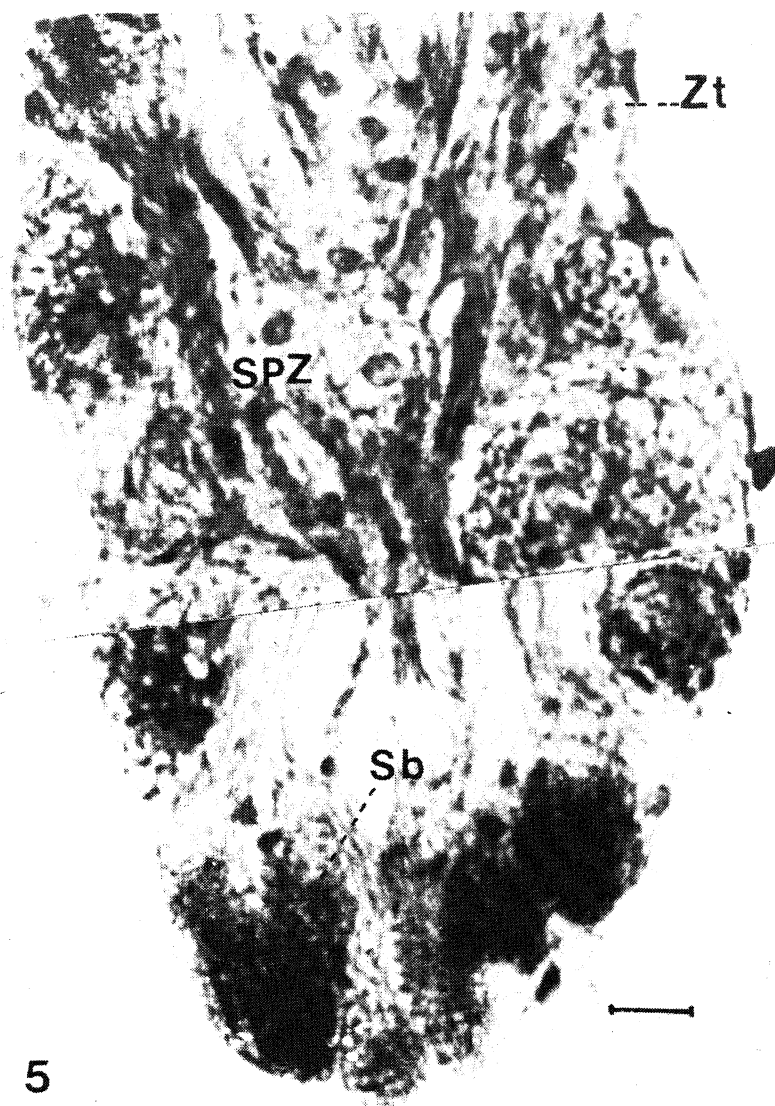


Figure 5. Enlarged view of the distal end of the testis showing Spz and sperm bundles (bar = 10 μ m).

Ultimately these Spz tend to aggregate into distinct sperm bundles, moving toward the head of the vas deferens (VD). Such sperm bundles are clearly seen in the distal end of the follicle (figure 3) sometimes inside the VD also (figure 1). In 2 globules (figure 3) of varying sizes presumably involved in sperm nourishment, are located in the centre of the follicle. The VD connects the follicle which is made up of single layer of epithelium, showing cuboidal epithelial cells, with large oval nuclei.

3.1 Ultrastructure

Spermatogenesis is evident in the adult testes of *A. ramakrishnae* in the early or young spermatid cyst, each cyst with a number of eupyrene sperm cells (10–25) varying from cyst to cyst (figure 6). These eupyrene sperm cells in the cyst are

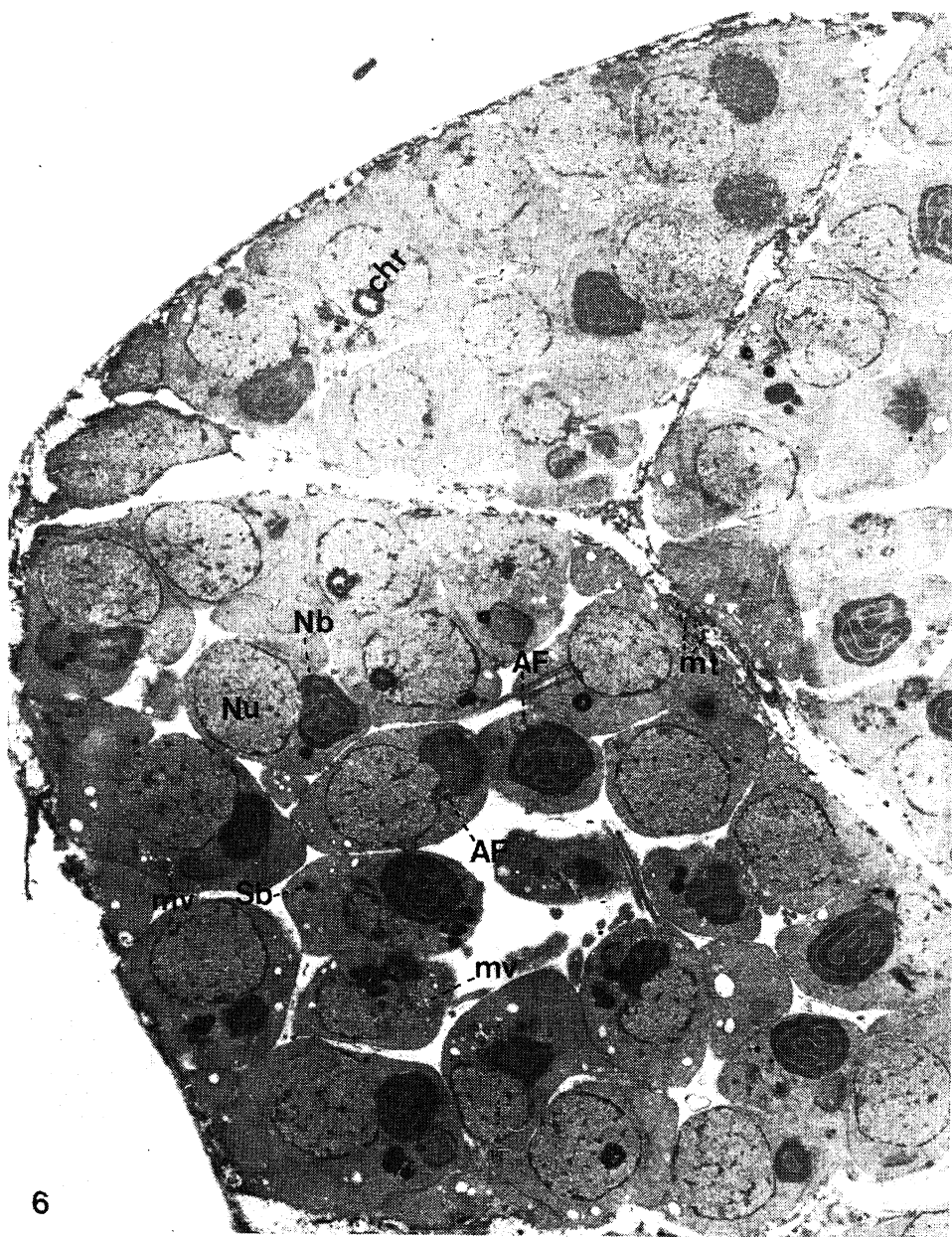


Figure 6. Developing cysts and the formation of axial filament and nebenkern ($\times 8500$).

embedded and interconnected by cytoplasmic bridges or cytoplasm. In the young spermatid cyst, each cell has a large nucleus with condensed chromatin material (figure 6). Cytoplasm of the cyst cells contains numerous mitochondrial fractions (figure 6). Multivesicular bodies are also common in the cytoplasm of spermatids (figure 6). The mitochondrial fractions in the cytoplasm aggregate and form the nebenkern, located next to the nucleus of each cell (figure 6). Each spermatid has about 3 axial filaments mostly in between the nucleus and nebenkern (figure 6). Sometimes the position is altered around the nebenkern. In a later stage these



Figure 7. Developing spermatids with 3 axial filament and condensed nebenkern ($\times 5000$). Arrows indicate axial filaments.

interconnected spermatids detach from each other and become individual developing spermatids (figure 7). Each developing spermatid appears with 3 prominent axial filaments, with the nebenkern formation in a condensed stage (figure 7).

The developing spermatids tend to orient in a group. The condensed nuclear material left over by the developing spermatids during the process of detachment

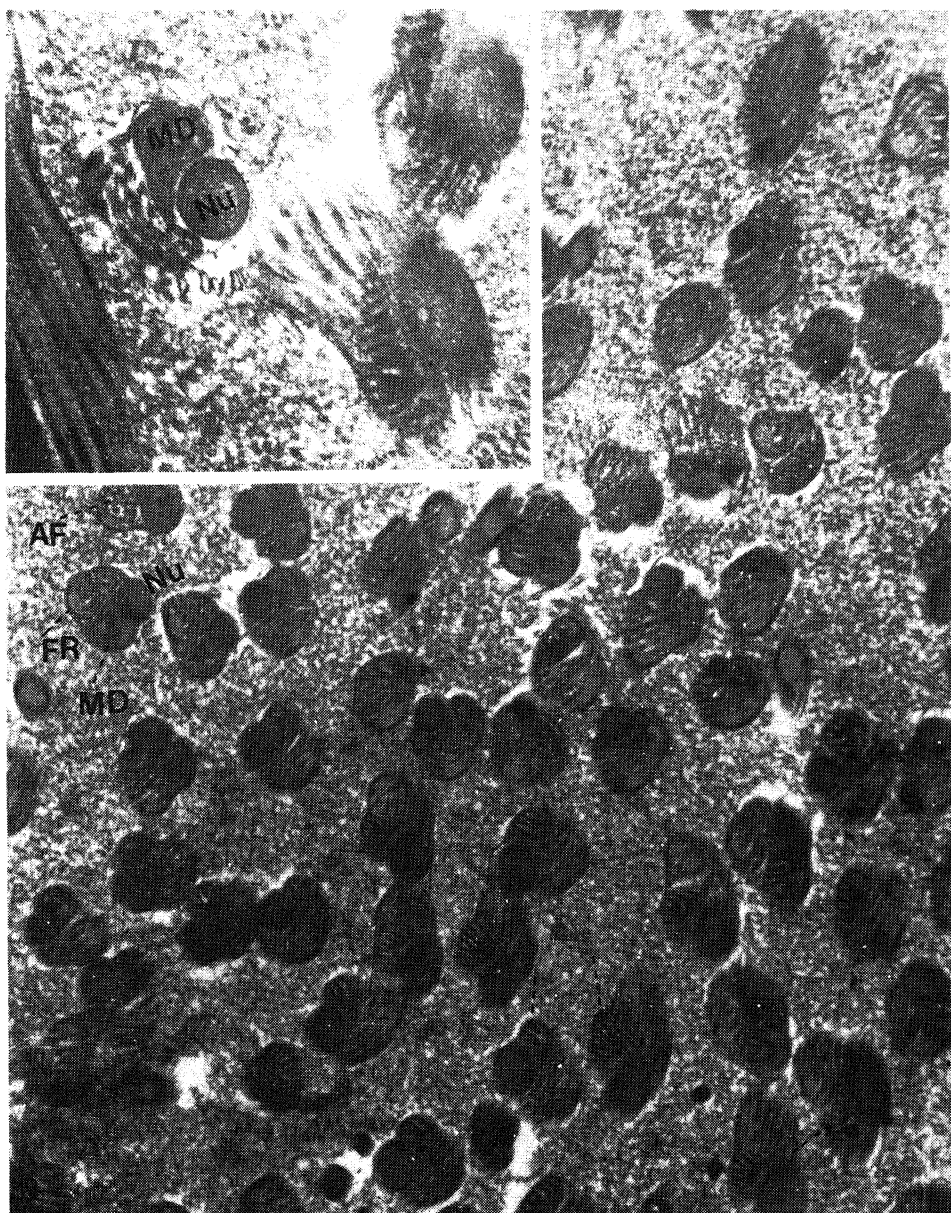


Figure 8. Number of spermatids with nucleus and MD. Inset: an enlarged view. Arrows indicate Cristae ($\times 37500$).

are discernible among the spermatids (figure 7). Later when the developing spermatids become transformed into spermatids, they begin to elongate and the nebenkern which was in a condensed stage in the developing phase, becomes elongated to form the mitochondrial body, conical in appearance (figure 8).

The cellular portion of the spermatid cells consists, in addition to the mitochondrial derivative (MD), a fibrous rod, above the MD, attached to the plasma membrane (PM). Three axial filaments, arranged in an irregular fashion and a dense globular nucleus, located along the side of the MD are evident (figure 8). The globular nuclei discernible in all the cells, continue to maintain their shape throughout spermatogenesis. During subsequent development the spermatids elongate further and become Spz (figure 9). During this period, MD also elongates, extending from the nucleus to the tail portion of the cell. Cristae are seen as parallel membranes that extend inward of the mitochondria (figure 8). The compactly arranged numerous round spermatids in each cyst which appears as honey comb structures, lose their contour and show spiral movement in the maturation phase (figure 9). During the process of elongation, each spermatid



Figure 9. Developing groups of spermatids and Spz ($\times 4600$).

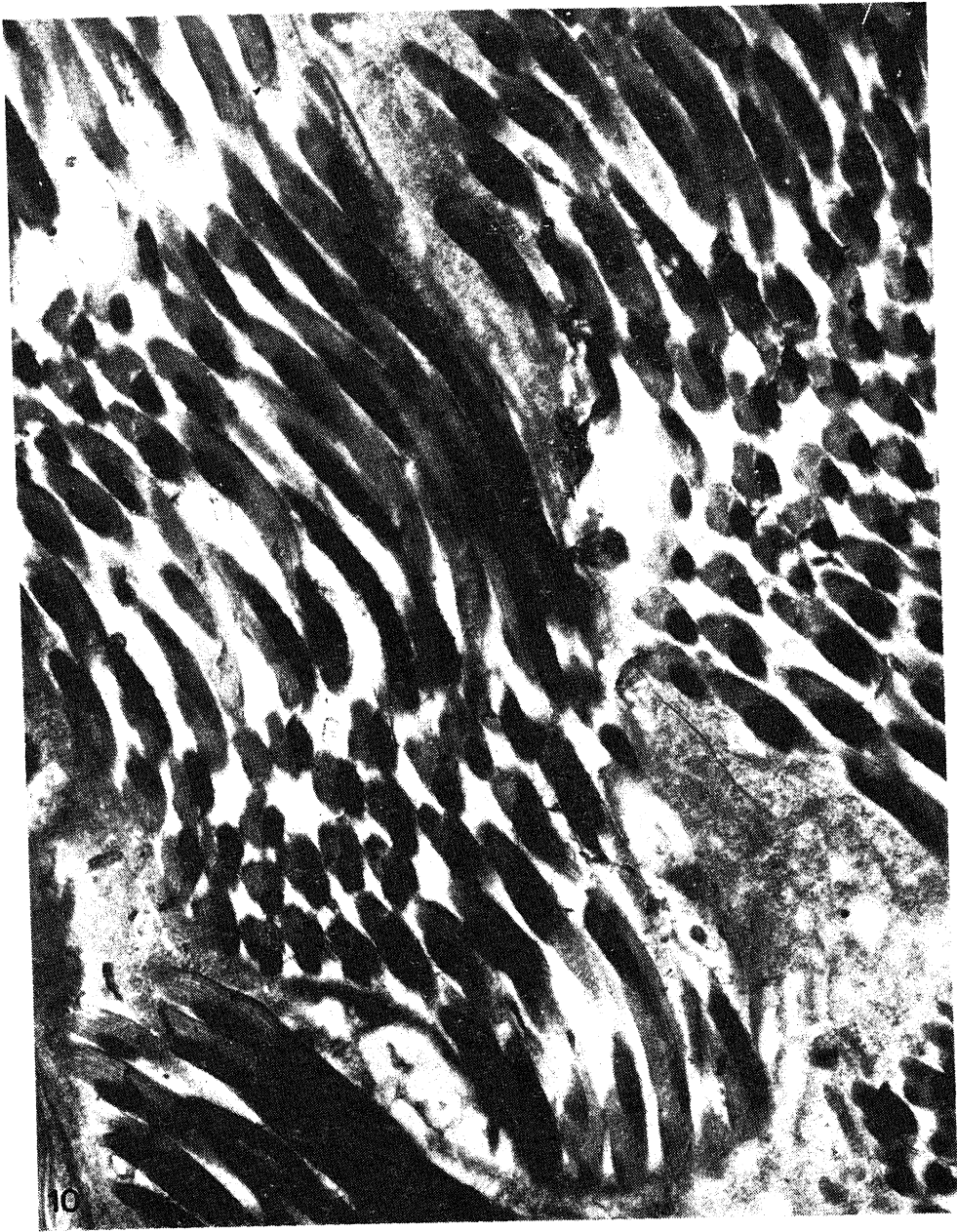
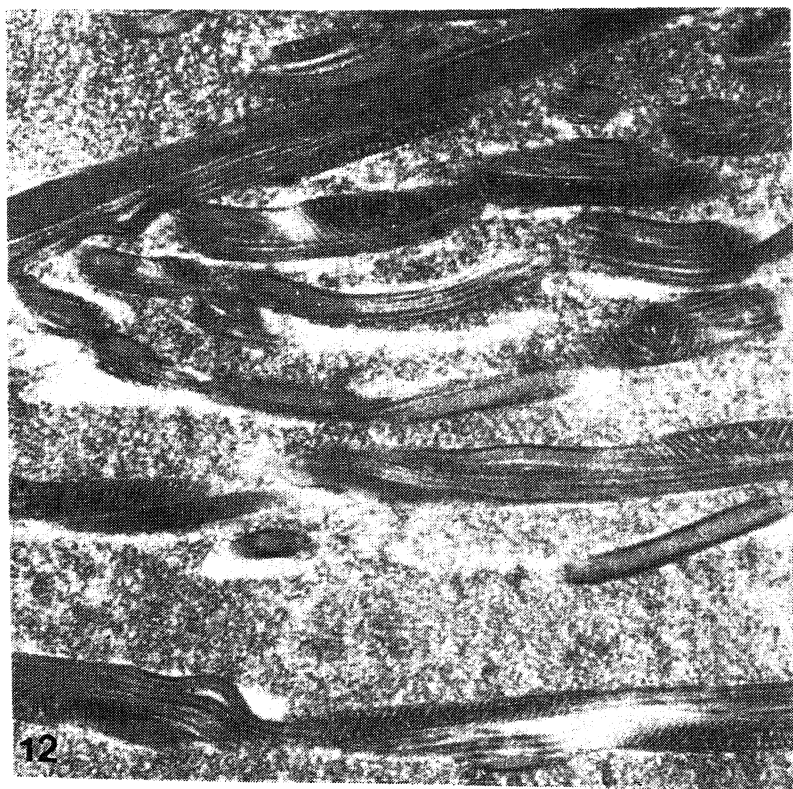
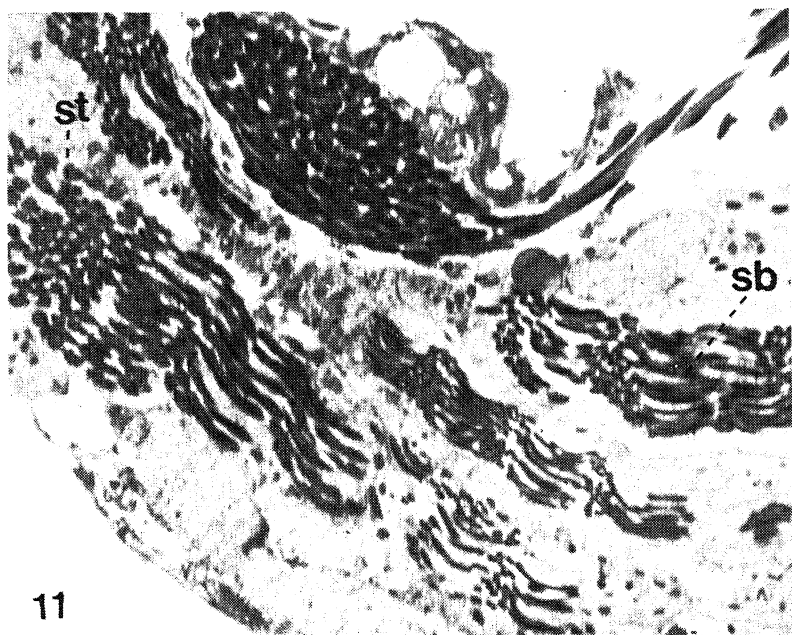


Figure 10. Enlarged view of the spermatids and Spz ($\times 12900$).

attains a length many times that of its width, becoming transformed into Spz (figures 11, 12). These Spz unite together and form sperm bundles.

4. Discussion

Basically, the development of sperms indicated in the present study involving



Figures 11 and 12.

different stages of spermatogenesis appear almost similar to that of other insects, except for minor differences in the development of the mitochondrial body. Though the formation of nebenkern appears as a common feature as observed in other insect sperms, it does not divide into two mitochondrial bodies, but instead is continued as a single body throughout spermatogenesis. This substantiates the view of Baccetti *et al* (1969), who indicated that the thysanopteran sperms have only one MD, with only one axial filament. However in the present studies the axial filament is observed to be 3 in number appearing as tiny structures near the nucleus and nebenkern in young spermatids, becoming more distinct as maturation progresses. A similar type of observation was made in the spermiogenesis of *Heliobius virescens* (Lanchance *et al* 1988) where the satellite body, an extracellular structure first appears as a tiny black speck on the PM of the immature eupyrene cells becoming much larger and more distinct and in more mature sperm cells becoming located near the axial filament and the MDs (Lanchance *et al* 1988). Such tiny black specks on the PM of immature spermatid cells are discernible in *Arrhenothrips* as well, but the subsequent development and the location of such a structure in the later stages of the sperm cells are not clearly identifiable.

The unusual features of the lepidopteran sperm, the regular production of both nucleate and anucleate cells and the presence of a very thick extracellular sheath around the nucleus are not observed in the present study except for the production of only eupyrene sperms. However, Bode (1983) reported the absence of a distinct acrosome and the elongated shape of nucleus in the sperm cells of *Thrips validus*, which are supposed to be apyrene sperms. Based on the number of flagella in the sperms of Thysanoptera, Bode (1989) indicated that the families of suborder Terebrantia are characterised by triflagellarity and only the family Phlaeothripidae is characterised by biflagellarity and that it must be the sister group of all other Thysanopterans. Interestingly enough the species *A. ramakrishnae* belonging to the family Phlaeothripidae is found to possess triflagellarity.

Multivesicular bodies are commonly present in the cytoplasm of spermatids. These bodies represent a type of lysosome and are reportedly involved in the uptake and digestion of protein and in cell lysis (Dingle 1972).

Acknowledgement

This work was possible during the tenure of the INSA Senior Scientists' award of the senior author (TNA). Thanks are due to Dr K. R. Seshan of the University of Texas, Austin, USA for his discussions and help in the preparation of the TEM figures.

Figures 11 and 12. 11. Groups of spermatids and sperm bundles ($\times 4600$). 12. Enlarged view of sperm bundles ($\times 27000$).

(Abbreviations used: ge, Germarium; Zg, zone of growth; Zm, zone of maturation; Zt, zone of transformation; Vd, vas deferens; Spt, spermatids; Spz, spermatozoa; Sb, sperm bundle; Sg, spermatogonia; PSc, primary spermatocyte; SSc secondary spermatocyte; Cy, cyst; Chr, chromatin material; Nb, nebenkern formation; Af, axial filament; mt, multivesicular bodies; Nu, nucleus; Nm, nuclear material; MD, mitochondrial derivative; FR, fibrous rod).

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Histological studies on female *Setaria digitata* (von Linstow 1906), a filaria of bovine, *Bos indicus*

S WILLIAM DECRUSE and R KALEYSA RAJ

Department of Biochemistry, University of Kerala, Kariavattom, Trivandrum 695 581, India

MS received 10 November 1989

Abstract. The fine structure of *Setaria digitata* based on histological assessment is described. The cuticle is thick and multilayered. The well developed longitudinal muscles consists of fibrillar zone only and cover about 65–70% of the circumferences throughout its body, the rest being occupied by the very voluminous lateral hypodermal chords. The digestive system is well developed and the intestine possesses a narrow simple tube consisting of a single layer of epithelial cells. The paired genital tubes run through almost the entire length of the body until the two uteri unite to form a short unpaired vagina. Morphologically distinct spermatheca is absent, but posterior part of uterus serves the function. Gradation of developmental stages—fertilized egg to microfilariae—occur from posterior to anterior part of uterus. The developing embryos are interconnected and also connected to the uterine wall. This condition commences from early embryogenesis and exists up to hatching stage. Probable function of these connections in the synchronised development of embryos and as 'nutritive channels' for the supply of nutrients has been discussed.

Keywords. *Setaria digitata*; filariae; embryonic interconnections; spermatheca; nutrient channels.

1. Introduction

Setaria digitata (von Linstow 1906) has been known for many years (Boulenger 1921; Thwaite 1927; Sheng 1958) but it is not satisfactorily described in the literature. *S. digitata* is a filarial parasite inhabiting the peritoneal cavity of cattle. Its general features had been reviewed by Sheng (1959). The species was first described by von Linstow (1906) as *Filaria digitata* from *Bos indicus* in Colombo. He had shown that it possesses rounded tail and later it was considered as one of the important distinctive characters. A few years later Raillet and Henry (1911) moved the species to the genus *Setaria*. The species *S. digitata* closely resembles *S. cervi* described by Maplestone (1931) from *Cervus axis* in the Zoological Garden, Calcutta (Sheng 1959). Even *S. cervi* has not been described in detail. Differentiation of both the species was demonstrated by Sheng (1958).

The parasite which had been described (Sheng 1959) possess a thick smooth cuticle and has a length of 65–75 mm and a maximum breadth of 0.5–0.7 mm. The oesophagus is 6–7 mm long, the anterior part measuring 0.5–0.6 mm and the posterior part 5.5–6.5 mm. The vulval opening is 0.5–0.6 mm from the mouth. The tail ends in a terminal spherical knob which may be perfectly smooth or slightly roughened with a papillated surface. The lateral caudal appendages are well developed. However, except these external features no information about its histological details are available.

As this species is being used as a model system for study of filariasis in this laboratory and due to lack of clear details especially of its female reproductive

system which has now become a focus of detailed biochemical and immunological studies, a detailed study of the structural features of *S. digitata* has been carried out.

2. Materials and methods

Adult *S. digitata* were collected from freshly slaughtered cattle in Tyrode solution. Healthy adults were fixed in Zenker's fluid overnight. The fixed worms were washed in repeated changes of 70% alcohol until it became colourless, and rapidly dehydrated by passage through graded ethanol, cleared in methyl benzoate and then embedded in celloidin and paraffin. Celloidin, 1 and 3% (w/v) in methyl benzoate and paraffin with 20% (w/w) Bee wax were used for embedding. The first embedding was carried out in 1 and 3% celloidin for 24 and 48 h respectively at room temperature. Second embedding was carried out in paraffin-Bee wax mixture at 60°C.

Paraffin blocks were prepared and sections cut at 8 μ m thickness using a rotary microtome. The sections were spread on glass slide coated with Mayer's albumin, de-waxed in xylene, dehydrated by passage through descending series concentration of ethanol to distilled water, stained in Harris' haematoxylin and counterstained with eosin. Standard histochemical techniques (Pearse 1968) were used throughout the procedure. The stained sections were dehydrated in ethanol series, cleared in xylene and mounted in DPX. The whole reproductive system and digestive system were dissected out and whole mount prepared for studying external morphology of both systems. Structural studies were carried out by optical microscope.

3. Results

3.1 Cuticle

Cuticle was observed as 4 layered with thick and unstained outer layer followed by intensely stained and faintly stained middle layers and the intensely stained basement layer (figures 1, 2).

3.2 Hypodermis or subcuticular layer

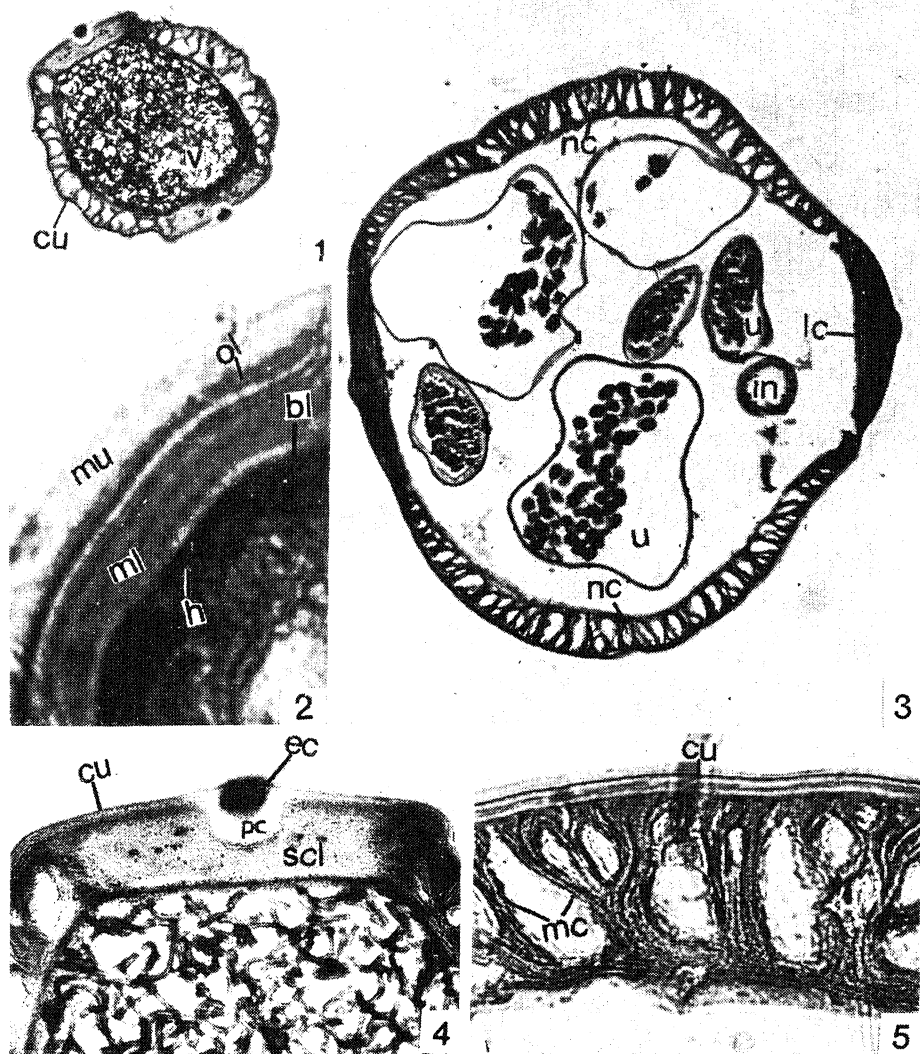
Hypodermis was observed as a thin layer beneath the cuticle and was characteristically thickened in the dorsal, ventral and lateral positions (figure 3) to form 4 hypodermal chords. The lateral chords were very prominent and protrude into the pseudocoelomic cavity between the somatic muscle.

3.3 Lateral line

Lateral line possess a flat structure with a canal of the excretory system passing through its centre (figures 1, 4). The suspensory cell layer, a distinct part stained with haematoxylin and projection of cuticle were clear (figure 4).

3.4 Somatic muscle

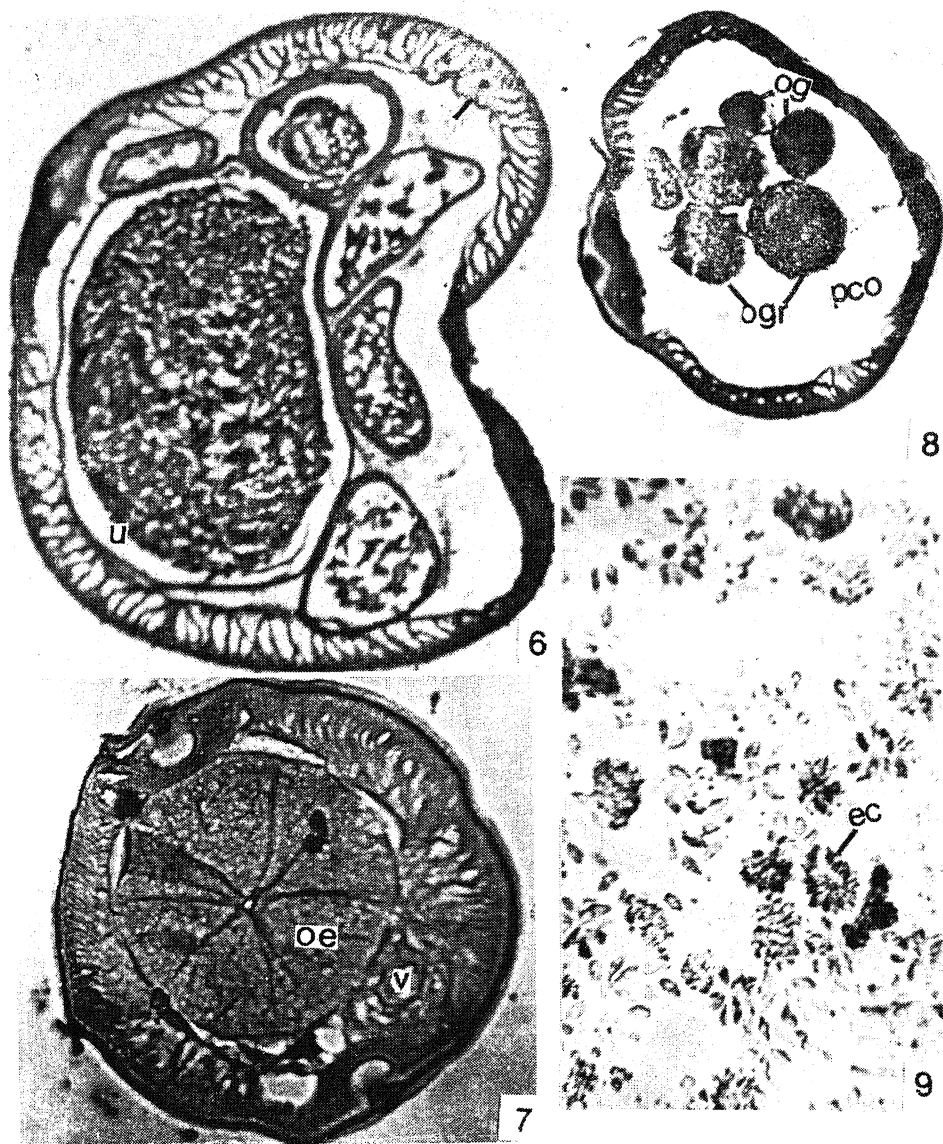
Somatic muscle was observed as a thick layer beneath hypodermis and the lateral hypodermal chords divide them into two major fields each with large number of



Figures 1-5. 1. TS of *S. digitata* through vagina (75:1). 2. TS of cuticle, a small portion of figure 1 (2000:1). 3. TS of mid-body region, broader posterior part of uterus contains developing embryos, narrow anterior part of uterus contains cross sections of mf (200:1). 4. TS of lateral line (250:1). 5. TS of muscular layer, narrow longitudinal muscles possess only fibrillar zone (1000:1).

(bl, Basement layer; cu, cuticle; ec, excretory canal; hy, hypodermis; in, intestine; lc, lateral hypodermal chord; m, muscular layer; mc, muscle cells; ml, thick middle layer; mu, outer mucous layer; nc, nerve chord; ol, outer thin layer; pc, projection of cuticle).

muscle rows i.e. polymyarian type (figure 3). The muscle cells were very narrow with one surface applied to the hypodermis and the other tapering surface, projecting into the pseudocoelomic cavity. Clear distinction of muscle cells into fibrillar and protoplasmic part was not evident (figure 5). Neuromuscular processes were very prominent, interconnected and directed towards nerve chord (figure 6).



Figures 6–9. 6. TS through sub-anterior part of body, broader anterior part of uterus and narrower sub-anterior part of uterus consists entirely of mf, neuromuscular processes; very prominent and directed towards nerve chords (200:1). 7. TS of anterior part of body through oesophagus (200:1). 8. TS of posterior part of body through ovary (200:1). 9. Egg clusters, growing eggs clustered around a central axis (200:1).

(ec, Egg cluster; in, intestine; nc, nerve chord; ne, neuromuscular connections; oe, oesophagus; og, ovary germinal zone; ogr, ovary growth zone; pco, pseudocoelomic cavity; u, uterus; v, vagina).

The longitudinal muscle layer cover about 65–70% of the circumference through almost entire length of the body, the rest being occupied by the very voluminous lateral hypodermal chords.

3.5 Digestive system

Digestive system is well developed in *S. digitata*. Its general morphology as reviewed by Sheng (1959) having distinct mouth, pharynx, intestine and anus. Pharynx possess centrally a narrow lumen surrounded by muscular tissue (figure 7). The intestine is a narrow simple tube consisting of a single layer of epithelial cells.

3.6 Female reproductive system

Didelphic (figure 15) type of female reproductive system was observed in *S. digitata*. Vulva beside mouth was surrounded by broad cuticular ring. Uncoiled vagina (15–20 mm) passes into two opisthodelphic and coiled uteri with narrow middle region and broader anterior as well as posterior regions (figure 15). Oviduct was very shorter (5 mm) and narrower. Telogonic ovary, 30–40 mm length ends blindly at tail region of the parasite and divided into growth and germinal zones. Short germinal zone was at the free end of the ovary and the presence of central rachis was not evident (figure 8). Spindle shaped oogonial cells at the growth zone were arranged freely in clusters around a central axis (figures 8, 9). The oogonial cells were more oval at the anterior part of ovary near oviduct.

Morphologically distinct spermatheca was absent, while posterior part of the uterus adjacent to which it meets the oviduct was broader and observed as sperm storage region (figure 15). Beyond the sperm storage region there was a gradation of developmental stages from early embryos at posterior part of uterus (figure 10) to fully embryonated eggs (figure 11) at middle part of uterus. Accumulation of microfilariae (mf) could be seen at the anterior part of uterus (figure 6) and in vagina (figure 1).

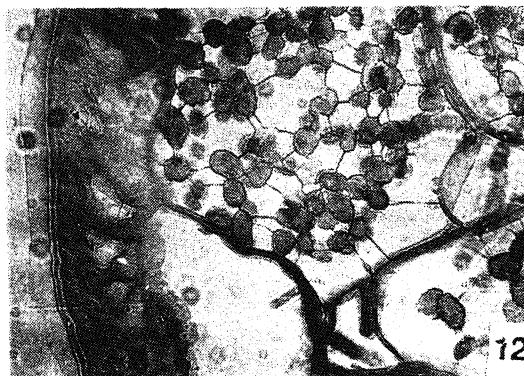
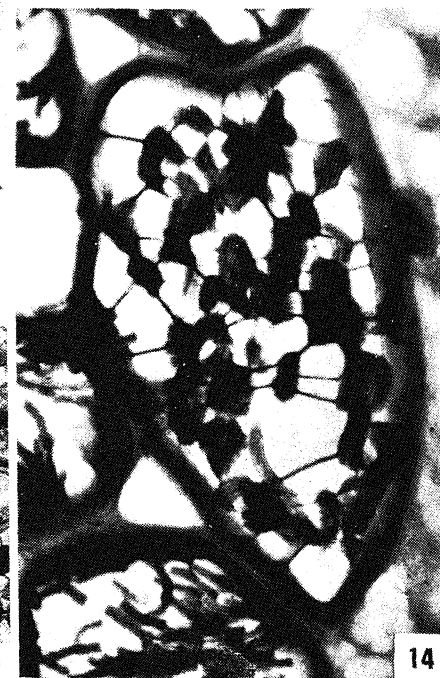
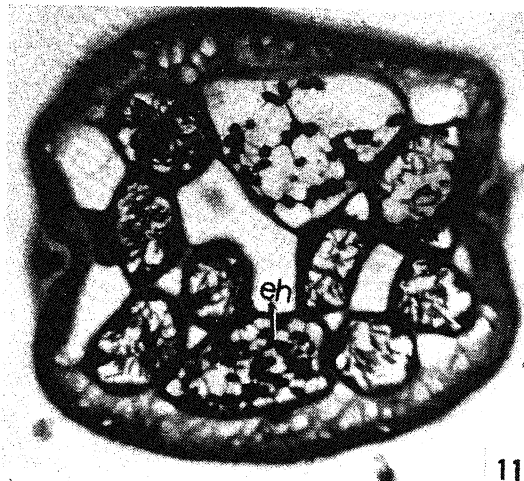
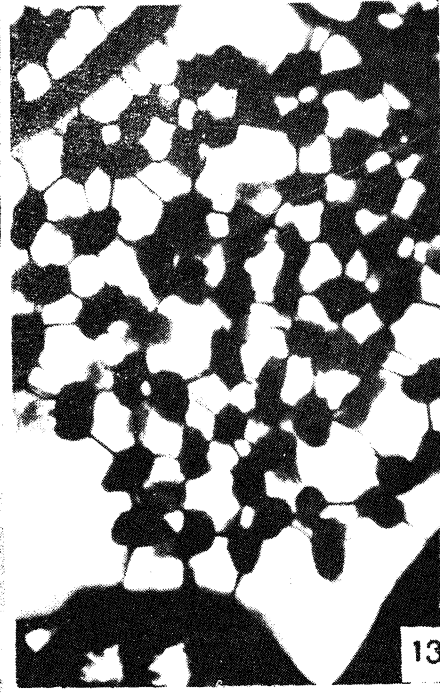
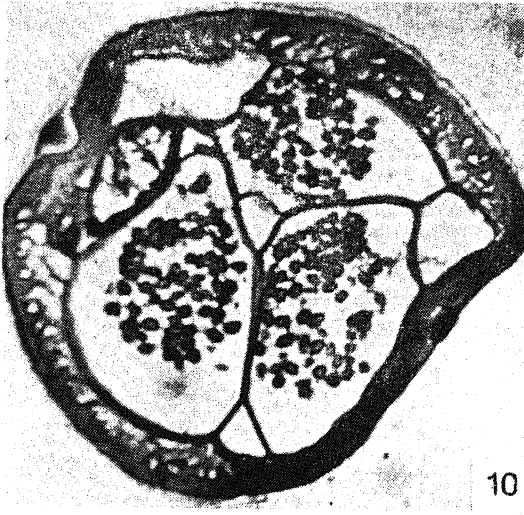
Embryos during development were not freely suspended in the uterus, but were interconnected and connected to the uterine wall. This condition was observed in early embryos (figures 12, 13) and upto hatching stage (figure 14).

4. Discussion

The species *S. digitata* was first described by von Linstow (1906). Its morphological structure had been re-described by Boulenger (1921) and reviewed by Sheng (1959). However, these reports were based on the values of measurements of worms and external structures. But detailed histological structure of the parasite was missing and hence the detailed histochemical study has been carried out to know the structure of female reproductive system.

The species described here was identified by London School of Tropical Medicine as *S. digitata*. The presence of similar external features in this species as that described by Sheng (1959) justifies their identity, eventhough the average size of the worm was little higher (70–90 mm) than the earlier report (65–75 mm).

The cuticle had been shown to possess 5 layers in *S. cervi* (Kagei 1960a, b) and 7 layers in *Litomosoides carinii* (Kagei 1963) under electron microscope, while both of these filarial parasites showed two layers under optical microscope. Eventhough the cuticle of *S. digitata* was observed as 4 layered (figure 7) it appears to possess still more layers (figure 2) with optical microscope. As the nematode cuticle appears



Figures 10-14. For caption, see page no. 112.

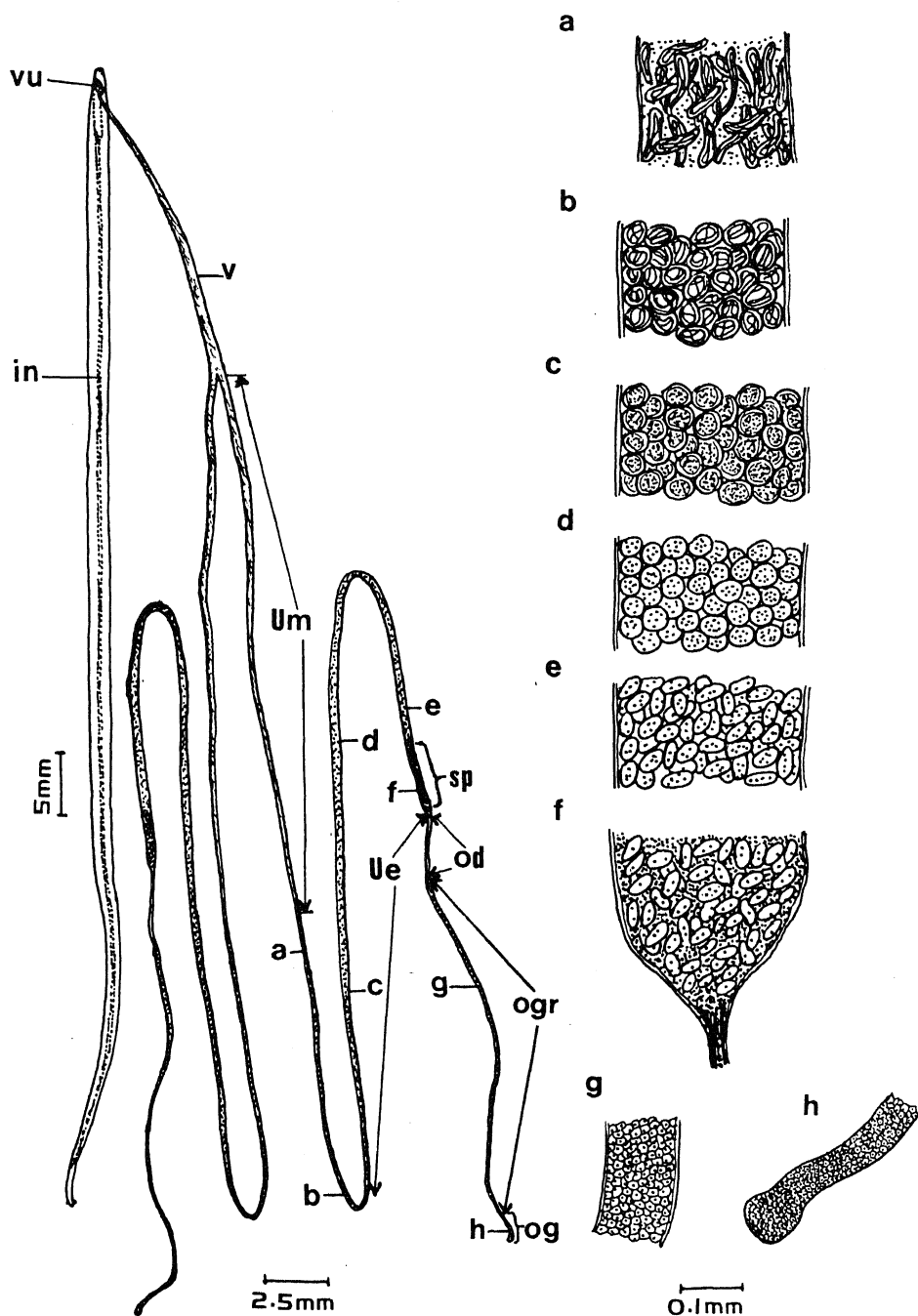


Figure 15. Diagram showing female reproductive system of *S. digitata*, vagina and anterior part of uterus consists entirely of mf.

(vu, Vulva; in, intestine; v, vagina; Um, uterus mf zone; Ue, uterus embryo zone; sp, spermatheca; od, oviduct; ogr, ovary growth zone; og, ovary germinal zone).

generally multilayered (Lee 1972) *S. digitata* should also possess the generalized structure.

Lateral line with the optical microscope consists of a characteristic structure which resembles that reported in *L. carinii* (Kagei 1963). The part stained with haematoxylin in the central of suspensory cell layer in *L. carinii* consists of a tube (Kagei 1963). In *S. digitata* the haematoxylin stained region in the central of suspensory cell layer and projections of cuticle as in *L. carinii* (Hyashi 1959; Kagei 1963) is evident. The carrying cell layer as described by Hyashi (1959) has not been observed in *S. digitata*.

The hypodermis of the simplest forms of nematodes has been described as being cellular by Chitwood and Chitwood (1950). In the more complex forms however, the hypodermis is usually syncytial (Bird and Bird 1969; Bird 1971) particularly in adults. Similar condition appears to be existing in adult *S. digitata* which is also a complex form of nematode.

The muscular layer of cattle filaria *S. cervi*, has been reported (Kagei 1960a, b) to be divided into contractile fibrillar zone and non-contractile protoplasmic zone (coelomyarian type). While, in the closely related cattle filaria *S. digitata* no such differentiation could be recognized (figure 5), but appears to be completely fibrillar (circomyarian type) as reported in cotton rat filaria *L. carinii* (Kagei 1963). Both these types are frequent in nematode somatic muscle (Bird 1971). It is quite clear (figure 6) that the muscle cells of *S. digitata* are interconnected in a complex manner. This arrangement was postulated to be necessary for efficient muscular co-ordination, particularly in the larger nematodes (Bird 1971).

Didelphic and opisthodelphic type of reproductive system observed in female *S. digitata* is a feature noted in many filarial parasites like *Filaria martis* (Anderson 1960), *Splendidofilaria* spp (Bartlett and Anderson 1985) and *Oncocerca volvulus* (Striebel 1988). The opening of vulva at anterior part of the parasite is characteristic feature of the genus *Setaria* (Sheng 1959). The elaborate reproductive system having a length of 135–195 mm, while the worm having 70–90 mm in length make the worm an mf producing machine.

The germinal zone of the telogonic ovary in *S. digitata* is short as in *Meloidogyne javanica* (Hirschmann 1971) and *Anguina tritici* (Triantophyllou and Hirschmann 1966). This is the region of active proliferation and consequently generate oogonial cells. At the growth zone, the oogonial cells increase in size and become spindle shaped as in *Filaria martis* (Anderson 1960).

Structurally distinct spermatheca has been well established in many nematodes like the filarial parasite *F. martis* (Anderson 1960) and in the plant parasitic nematodes *M. javanica* (Hirschmann 1971) and *A. tritici* (Triantophyllou and Hirschmann 1966). Eventhough such a structurally distinct spermatheca was absent in *S. digitata*, a region of uterus adjacent to where it meets the oviduct performs the same function.

Since at the sperm storage region sperms could be seen intermingled with eggs, it is suggested that fertilization is taking place at this region. After fertilization the eggs undergo embryogenesis and finally a fully embryonated egg is produced. The region of transition of embryos to mf observed at the middle part of the narrower region of the uterus indicates that hatching process is taking place at this region. Release of young juveniles is a peculiarity of filarial parasites, and sometimes they are referred as 'viviparous'. *S. digitata* releases large number of mf and so belongs to the 'viviparous' group.

The interconnections of developing embryos and their connection with uterine wall is a new observation made in *S. digitata* (figures 13, 14). This peculiarity noted in *S. digitata* has not so far been observed in any other nematode including the same genus. Eventhough these connections some how, resembles that of vascular connections of mammals and some other vertebrates (Balinsky 1976), which are advanced animals, the function of such connections in this primitive class is not clear. In the human filarial parasite *O. volvulus*, nutrient channels extending from uterine wall to the embryos has been proposed to be present (Schulz-key 1988) eventhough such channels were not observed in the histological examination (Buttner *et al* 1988). Whereas, in *S. digitata* the embryonic interconnections observed are similar to that of the proposed channels in *O. volvulus*, and likely to function as 'nutrient channels'. In addition, synchronised development and systematic distribution pattern of developing embryos inside uterus may be due to physical support provided by these connections so that various embryonic stages are arranged sequentially and located inside the genital tract at its specific level. However, it provides additional points to consider the species under viviparous group.

The general morphology of the parasite in many respects agree with the details provided for filarial parasites such as *O. volvulus*, *L. carinii* and *S. cervi*. However, the present study provide additional information on the morphological features and in addition points out the differences. The embryonic interconnections noted in the reproductive system is very peculiar since such structures have not been observed so far. If it possess the function of nutrient circulation resembling placenta in mammals, it will carry some evolutionary significance like co-evolution of the 'viviparous' nematode with advanced viviparous mammalian host. However, an exact function of these connections have to be understood before any conclusion could be drawn.

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Figures 10–14. 10. TS of posterior part of body, broader posterior part of uterus consists of embryos at early stages (200:1). 11. TS of middle part of body, middle part of uterus (broader) consists of fully matured embryos interconnected and connected to uterine wall, narrow anterior part of uterus consists entirely of microfilariae (the embryonic interconnections are missing) (200:1). 12. TS through posterior part of uterus, developing embryos interconnected and connected to uterine wall (375:1). 13. TS of posterior region of uterus, more advanced stage than that of figure 12 (675:1). 14. TS of middle part of uterus, fully matured embryos still interconnected and connected to uterine wall (675:1).

(eh, Embryos at hatching stage).

Influence of neem kernel extract on morphogenesis and vitellogenic oocyte development in *Trogoderma granarium* everts

S CHELLAYAN and G K KARNAVAR

Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581, India

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Abstract. Neem seed kernel extracts adversely affect the growth and morphogenesis of insects. In *Trogoderma granarium*, neem seed kernel extract was found to inhibit normal pupal-adult development. The malformed adults comprised a heterogenous group of transitory forms between the pupal and adult types. Older pupae were less sensitive to the compound. In the seemingly normal surviving adults of *Trogoderma granarium* a reduction in the number of vitellogenic oocytes was found. The active compound(s) in the neem seed kernel extracts is proposed to produce the observed effects by regulating morphogenetic hormone titres, though the precise mode of action cannot be safely defined.

Keywords. *Trogoderma granarium*; neem seed extract; morphogenesis; vitellogenic oocytes; juvenile hormone.

1. Introduction

The active principles in neem have been identified to be tetranortriterpenoids (Krause and Adami 1984). These adversely affect the growth and morphogenesis of insects (McMillian *et al* 1969; Steets 1975; Meisner *et al* 1976, 1978; Saxena *et al* 1981; Schmutterer 1985). In neem seeds, azadirachtin has been found in the highest concentration and was therefore considered to serve as a substance representative of the biological activity (Schneider and Ermel 1987). However, Schmutterer and Zebitz (1984) indicated that other compounds also exert synergistic or antagonistic effects on insects.

Comparatively little information is available on the influence of neem seed kernel extracts (NKE) on the development of vitellogenic oocytes in insects. Schulz and Schluter (1984) revealed the histopathological effects of NKE on egg maturation in *Epilachna varivestis*. They had reported degenerative changes in the oocytes and disturbances in yolk deposition during vitellogenesis. Related to these degenerative changes, significant disruptive changes in the corpus allatum and alterations in the corpus cardiacum and neurosecretory cells in the pars intercerebralis are established.

The growth disruptive action of methanolic NKE was studied in a stored grain pest, *Trogoderma granarium*. The influence of NKE on the development of vitellogenic oocytes was also examined. In *T. granarium*, the development of the oocytes occurs during the late pupal period and the adults emerge with full complement of eggs. The adults are short lived and do not feed. *T. granarium* is therefore an ideal insect to study the influence of NKE on oocyte development. Oocyte development of seemingly normal adults produced after NKE treatment is reported here.

2. Materials and methods

T. granarium (Coleoptera; Dermestidae) was maintained on crushed wheat at

$35 \pm 1^\circ\text{C}$. Fifth instar larvae were collected and maintained separately for pupation. Pre-pupae roll slightly and lie on their back, motionless for a few hours. Afterwards their body becomes stretched and a split of the larval cuticle appears on the dorsal side. This marked the initiation of pupation and such pupae were taken as 0 h old.

Neem kernel was extracted with methanol repeatedly and the solvent evaporated off. Known quantity of the residue was dissolved in methanol and topically applied ($1 \mu\text{l}/\text{pupa}$) to the pupae (10/test/concentration/replicates) with a Hamilton microsyringe. Four concentrations—10, 20, 50 and $100 \mu\text{g}$ per μl per pupa were applied on 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90 and 96 h old pupae. Methanol treated ($1 \mu\text{l}/\text{pupa}$) pupae served as controls. After treatment, pupae were maintained for adult emergence. At the end of the normal pupal period, the individuals were examined for any abnormalities and a record of normal and abnormal adults was kept.

In order to study the vitellogenic oocyte development, ovaries were dissected from seemingly normal adults within 24 h of emergence and examined under the microscope. The number of vitellogenic oocytes in each female was determined. As small adults produce lesser number of eggs (Karnavar 1972), pupae of the same size and weight served as experimental and control (20 insects/batch; 17 batches were used). The transformed (square root) data of the vitellogenic oocyte number were analyzed using an analysis of variance (ANOVA), followed by multiple comparison procedures using the least significant difference (LSD) procedure (Ott 1984). The data were transformed in order to fulfill the assumption of normally distributed observations for statistical tests.

3. Results

3.1 Influence on morphogenesis

The per cent of abnormal adults obtained is presented in figure 1. The malformed adults comprised a heterogeneous group of transitory forms between the pupal and adult types. Based on the extent of malformations, the individuals were differentiated into 6 grades (Karnavar 1973a). In grade-I forms the morphogenetic aberrations were observed to be maximum. Grade-II individuals showed morphogenetic development slightly advanced compared to the grade-I forms. The fore and hind

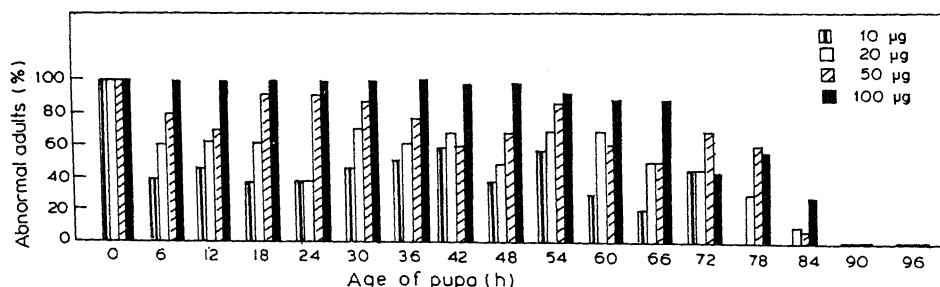


Figure 1. Per cent of abnormal adults derived from whole neem extract treated *T. granarium* pupae.

wings were slightly curled away from the body. The hind wing buds were observed to be swollen and filled with a fluid. In grade-III forms, tanning was observed on the entire anterior ventral and on the head region. The development of the wings and the legs was not complete. Grade-IV individuals showed morphogenetic development very much advanced. The wings were not fully formed. The legs were well developed. The adults were capable of independent movement. In grade-V individuals, adult characteristics were predominant. Grade-VI individuals were normal-looking adults with wings imperfectly developed. The adult-like forms were able to move about and mate. Pupae in the later stages of development i.e. 84, 90 and 96 h old are relatively insensitive to the NKE treatment. In all 4 dosages, 70–100% morphologically normal adults were obtained from 84 h pupa, whereas in 90 and 96 h pupa, adult development was 100%. All the control pupae developed normally.

3.2 Influence on vitellogenic oocyte development

Table 1 shows the number of vitellogenic oocytes in treated and control insects.

The ANOVA of the vitellogenic oocyte number (table 2) show that statistically significant ($\alpha \leq 0.05$) differences exist among the factors age (17 levels; 0 through 96 h) and treatment (2 levels; control and experimental). The effect due to the different concentrations (10, 20, 50 and 100 μg) was not statistically significant indicating that the concentration does not affect the growth of eggs. All the two-way interactions between the factors (age \times treatment, age \times concentration, and treatment \times concentration) were significant. However, the 3-way interaction was not significant.

4. Discussion

In *T. granarium*, NKE was found to inhibit normal pupal-adult development. The resulting adultoids were of several types, showing preservation of pupal features to different extent. Older pupae were found to be less responsive. Distinct morphogenetic disorders, following treatment with neem seed extracts have also been reported in *Manduca sexta* (Haasler 1984) and *Antestiopsis orbitalis bechuana* (Leuschner 1972). The insect growth regulating effects of neem compounds have been explained to result from disturbances in the endocrine system. In the present studies with NKE, the characteristics of pupal-adult intermediates obtained resembled those obtained from *T. granarium* following application of juvenile hormone analogues (Karnavar 1973a). Maintenance of a high juvenile hormone titer during metamorphosis was interpreted to cause the disruption of development in neem seed extract-treated *M. sexta* fifth instar larva (Haasler 1984). Juvenilizing effect of neem kernel suspension was also reported in *Chilo partellus* and *Heliothis armigera* larvae (Jotwani and Srivastava 1984). In *Aedes aegypti* also the impairment of pupal and adult development was related to changes induced by synthetic juvenoids such as altosid in the insect (Zebitz 1984). In *T. granarium* also, a disturbance in the hormone titers could be generalized to result from NKE application.

In the seemingly normal surviving adults of *T. granarium*, a reduction in the number of vitellogenic oocytes was observed. A dose-related effect was not found,

Table 1. The effect of whole neem extract on the vitellogenic oocyte number in *Trogoderma*.

Age of pupa (h)	Dose of whole neem extract ($\mu\text{g/pupa}$)									
	10 μg		20 μg				50 μg		100 μg	
	Number of vitellogenic oocytes per female (mean \pm SD)									
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
0	60.0 \pm 12.0	61.0 \pm 11.0	64.0 \pm 16.18	68.5 \pm 16.17	54.85 \pm 12.60	68.57 \pm 5.42	49.0 \pm 13.70	63.75 \pm 6.04		
6	68.75 \pm 13.98	75.5 \pm 5.07	63.77 \pm 11.33	71.11 \pm 4.63	57.77 \pm 21.74	74.29 \pm 4.54	42.57 \pm 16.48	73.71 \pm 4.59		
12	62.5 \pm 11.52	68.4 \pm 8.71	65.33 \pm 12.86	71.56 \pm 11.49	67.5 \pm 15.63	72 \pm 11.56	49.56 \pm 15.96	64.44 \pm 7.60		
18	58.44 \pm 12.99	68.44 \pm 9.18	45.25 \pm 11.70	70.0 \pm 8.72	45.33 \pm 14.94	70.67 \pm 7.83	53.0 \pm 23.17	75.0 \pm 5.19		
24	57.11 \pm 13.37	65.78 \pm 9.21	60.8 \pm 11.39	66.2 \pm 5.83	63.0 \pm 5.20	67.5 \pm 3.27	76.0 \pm 4.0	78.0 \pm 6.0		
30	64.5 \pm 12.68	72.0 \pm 84.9	57.14 \pm 14.61	67.14 \pm 5.64	61.25 \pm 12.37	63.75 \pm 10.93	47.6 \pm 15.81	74.4 \pm 4.8		
36	52.67 \pm 16.22	64.44 \pm 8.93	57.25 \pm 13.15	66.0 \pm 6.08	66.0 \pm 6.0	67.0 \pm 5.0	53.2 \pm 18.14	65.6 \pm 9.24		
42	44.0 \pm 5.29	63.0 \pm 7.21	42.67 \pm 8.84	59.67 \pm 7.52	50.0 \pm 6.32	61.5 \pm 6.38	38.0 \pm 7.48	64.67 \pm 5.24		
48	63.8 \pm 9.31	70.67 \pm 7.94	67.2 \pm 10.09	72.2 \pm 6.66	51.56 \pm 18.11	66.67 \pm 7.72	57.5 \pm 5.55	66.0 \pm 9.27		
54	55.56 \pm 14.04	68.44 \pm 9.46	48.22 \pm 9.82	64.89 \pm 6.40	60.0 \pm 14.80	75.0 \pm 5.20	44.29 \pm 11.58	72.57 \pm 6.57		
60	69.0 \pm 5.19	73.25 \pm 2.63	67.33 \pm 6.79	70.22 \pm 4.93	55.71 \pm 16.68	69.43 \pm 6.21	45.71 \pm 15.06	63.71 \pm 7.89		
66	56.67 \pm 12.36	65.11 \pm 5.74	53.0 \pm 12.57	64.0 \pm 5.48	54.0 \pm 12.42	68.29 \pm 8.31	62.0 \pm 1.0	67.25 \pm 7.28		
72	61.42 \pm 13.29	68.86 \pm 7.77	57.11 \pm 10.71	65.78 \pm 5.37	58.0 \pm 14.42	64.89 \pm 11.97	71.25 \pm 3.42	72.0 \pm 3.16		
78	63.2 \pm 10.13	69.6 \pm 6.92	58.4 \pm 13.99	67.6 \pm 8.85	50.25 \pm 14.05	64.75 \pm 7.87	63.6 \pm 15.54	72.4 \pm 9.24		
84	69.0 \pm 14.06	75.0 \pm 6.71	66.2 \pm 12.88	69.2 \pm 9.6	64.6 \pm 12.13	68.4 \pm 5.49	64.4 \pm 12.83	72.8 \pm 5.88		
90	59.0 \pm 10.96	71.8 \pm 11.58	55.4 \pm 13.8	67.8 \pm 5.25	63.2 \pm 9.09	72.6 \pm 12.71	67.2 \pm 14.15	78.4 \pm 7.63		
96	62.4 \pm 11.93	73.6 \pm 7.79	63.0 \pm 16.86	72.2 \pm 7.29	58.0 \pm 11.06	72.6 \pm 5.89	63.2 \pm 15.29	70.8 \pm 8.4		

Table 2. The three-factor ANOVA of the transformed oocyte number in *T. granarium* when crude neem kernel extract was used.

Source of variation	df	ss	F	PR > F
Age	16	52.6154	5.40	0.0001
Treatment	1	139.5381	243.26	0.0001
Concentration	3	3.1567	1.83	0.1392
Age × treatment	16	20.8765	2.27	0.0029
Age × concentration	48	60.0180	2.18	0.0001
Treatment × concentration	3	26.7013	0.97	0.5330
Age × treatment × concentration	48	26.7013	0.97	0.5330
Error	931	534.0363	—	—
Total	1067	841.6999	—	—

df, Degrees of freedom; ss, sum of squares; F, table value; PR, Probability.

showing that the concentration of neem extract does not influence the development of vitellogenic oocytes in this insect. However, significant differences exist among the factors, age of pupa and neem extract treatment (control and experimental). Comparatively few reports are available on the specific effects of NKE during the course of reproductive development. When fifth instar nymphs of *Dysdercus fasciatus* were treated with neem extracts, fecundity of the adults as well as the hatchability of the eggs were lowered, while topical treatment of third instar nymphs was shown to have no effect on the fecundity of resulting females (cf. Schmutterer 1984). Larvae of *C. partellus* that survived the neem kernel suspension treatment were reported to form abnormal adults, incapable of producing another generation (Jotwani and Srivastava 1984). Influence of various neem extracts on the development of the Egyptian cotton leaf worm *Spodoptera littoralis* larvae was studied by Ascher *et al* (1984) and strong fecundity-reducing effects in the females that survived the larval treatment was observed. In *Tetranychus cinnabarinus*, repellency and reduction of fecundity and also mortality of adults was observed when extracts were sprayed directly on adult female mites on bean leaf discs. Severe damage was noticed in the germarium and vitellarium zones of the ovarioles of *E. varivestis* females fed on NKE, in addition to effects on developing oocytes (Schulz and Schluter 1984). The effects were not found to depend on the dose of neem and a correlation between the time of treatment on one side and the physiological state of the individuals on the other was indicated. These reports show that the influence of NKE on the development of vitellogenic oocytes in *T. granarium* is not unusual. Schulz and Schluter (1984) investigated the inhibitory effects of neem on oocyte development in *E. varivestis* in some detail. They had found a decrease of protein concentration in the haemolymph and oocytes in the neem treated beetles. They also had described changes in the corpus allatum of 3 and 6 day old treated beetles which appeared to indicate lytic processes and alternations in the corpora cardiaca and the neurosecretory cells. In *T. granarium*, differences in oocyte and fat body protein concentration in females obtained from NKE treated pupa and controls was observed (S Chellayan and G K Karnavar, unpublished results). Oocyte protein composition was observed to be low in association with neem treatment while the fat body protein showed a higher level when compared to the controls. In most insects, precursors of the major yolk proteins are synthesized in the fat body, released into the haemolymph and

sequestered by the ovaries (Keeley 1978; Hagedorn and Kunkel 1979). Control of egg development is accomplished by hormones, primarily juvenile hormone and in certain species like *A. aegypti*, *Drosophila melanogaster*, ecdysteroids or both (Engelmann 1986). Concerning the results of the present experiments it may be assumed that the active compound(s) in the NKE produce the observed effects by regulating the titres of the morphogenetic hormones, though the precise action cannot be safely defined.

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Effect of plumbagin on haemocytes of *Dysdercus koenigii* F.

B P SAXENA and K TIKKU

Division of Insect Physiology, Regional Research Laboratory, Jammu 180 001, India

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Abstract. Effect of plumbagin, a phytochemical, on the haemocytes of *Dysdercus koenigii* was studied after topical application. Scanning electron microscopical studies showed deformity in surface morphology in almost all the 5 types of haemocytes categorized in the bug, especially that of granular haemocytes and plasmatocytes, which are devoid of their filopods in the treated insects. The fat droplets of adipohaemocytes shrink while oenocytoid is affected to a lesser degree. Plasma membrane of all the cells becomes fragile leading to a gradual loss of cytoplasm and ultimately only a few interconnected cytoplasmic strands are left. Ultrathin sections revealed a highly vacuolized condition and disintegrating organelles which pass out of the cells due to ruptures of very thin plasma membrane. Total and differential haemocyte counts performed after 24 and 48 h of treatment showed a drastic reduction of all the types i.e. the prohaemocytes disappear from blood, the number of granular haemocytes and plasmatocytes declines while oenocytoids and adipohaemocytes comprise the major part of counts. Because of the damages caused to haemocytes and the suppression of filopodial elongations of plasmatocytes and granular haemocytes (the types that are active in defense mechanism), it can be inferred that cellular defense reactions of *Dysdercus koenigii* are reduced after plumbagin treatment.

Keywords. Plumbagin; haemocytes; *Dysdercus koenigii*; electron microscopy; cellular reactions.

1. Introduction

Insect haemocytes are known to respond to biological agents as well as toxins by exhibiting various reactions like phagocytosis, encapsulation, disintegration or distortion in their contours, in accordance with the nature of substance by which they have been challenged (Feir 1979; Ratcliffe and Rowley 1979). Observation of gradual deaths in diseased condition within a period of 5–10 days after emergence in *Dysdercus koenigii* adults, exposed to a plant derivative plumbagin, led to a speculation that the compound might be interfering with the defense mechanism of the bug by increasing its susceptibility to diseases. Literature survey also reveals plumbagin to retard growth and cause mortality of nymphs in *D. koenigii* as well as *D. cingulatus* (Chadha *et al* 1986; Joshi *et al* 1988), in addition to inhibiting ecdysis of some lepidopterous larvae (Kubo *et al* 1983). Keeping this in view, the effect of plumbagin on the haemocytes of *D. koenigii* has been studied.

2. Materials and methods

D. koenigii adults reared under standard conditions (Saxena and Srivastava 1972), were given a topical application of 0.1% plumbagin (source: *Plumbago zeylanica*: pure compound) in acetone (2 μ l/individual) and total (THC) and differential (DHC) haemocyte counts taken (after 24 and 48 h) with simultaneous controls of pure

acetone. Scanning and transmission electron microscopical studies were conducted according to the methods of Sharma *et al* (1986) and Saxena *et al* (1988).

3. Results

D. koenigii contains 5 types of haemocytes as identified by light microscopy (unpublished results) viz. prohaemocytes, plasmatocytes, granular haemocytes, oenocytoids and adipohaemocytes. Plumbagin application showed a significant reduction in almost all the types (table 1). Figures 1–12 show the deformity in shapes of these cells. The haemocytes which generally act for defense i.e. plasmatocytes and granular haemocytes, mainly bear the effect because most of them either lyse, change their contour or become fragile and are on the verge of collapse. There is loss of pseudopods in plasmatocytes and a shrinkage in their cytoplasm (figure 4) while, due to filopodial retraction, the geometry of granular haemocytes is altered (figure 5) and in extreme forms only reminiscent of a cell, having interconnected cytoplasmic strands, is left (figure 9). The adipohaemocytes retain their fat droplets but on account of shrinking in the size of such droplets, a bizarre bunch-like appearance is imparted to the cell (figure 8). The least distorted type is oenocytoid,

Table 1. The DHC and THC before and after plumbagin treatment.

	24 h		48 h	
	Control* DHC	Treated DHC	Control* DHC	Treated DHC
Prohaemocytes (%)	11.60 ± 2.45	0.00 ± 0.00	6.10 ± 2.42	0.00 ± 0.00
Granular haemocytes (%)	36.90 ± 6.13	23.10 ± 5.36	37.00 ± 5.56	14.00 ± 5.92
Plasmatocytes (%)	31.80 ± 2.93	15.70 ± 5.17	33.50 ± 7.97	10.70 ± 5.79
Oenocytoids (%)	12.20 ± 5.57	30.00 ± 6.09	15.20 ± 3.96	37.80 ± 8.37
Adipohaemocytes (%)	7.50 ± 3.95	34.10 ± 4.53	10.10 ± 6.33	36.80 ± 6.16
Number of haemocytes per cubic mm (THC)	7000.00 ± 608.27	4360.00 ± 517.68	5860.00 ± 403.73	2780.00 ± 649.61

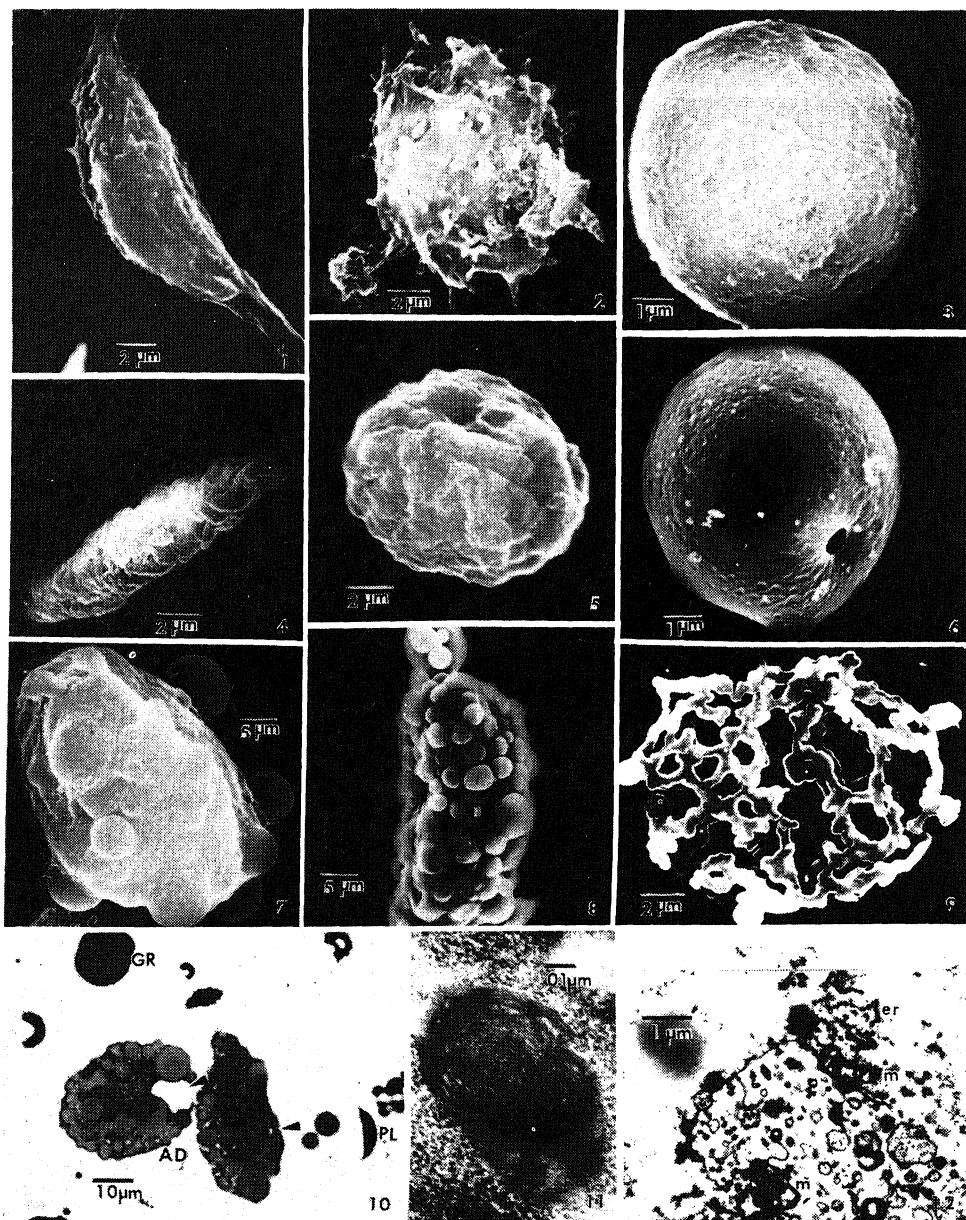
N = 10; % = number of particular haemocyte out of 100 haemocytes counted for each reading.

*Pure acetone.

Figures 1–12. Haemocytes of normal and plumbagin treated *D. koenigii*. 1–3 and 7. Normal haemocytes. 1. Spindle shaped plasmatocyte with extended pseudopods. 2. A granular haemocyte with cytoplasmic projections and filopods emanating from the body. 3. An oval oenocytoid with an almost smooth outline. 7. Large fat droplets protruding from the body of an adipohaemocyte. 4–6, 8 and 9. Plumbagin affected haemocytes. 4. A shrunken plasmatocyte. 5. A granular haemocyte with absence of all processes. 6. A punctured oenocytoid. 8. An adipohaemocyte with diminutive fat droplets. 9. Remnants of a granular haemocyte. 10. A toluidineblue stained semithin section showing some of affected haemocytes, note the degranulation of a granular haemocyte (GR) and an enhancement of vacuole formation (arrows) in adipohaemocytes (AD) (PL, plasmatocyte). 11. The structured granule of an affected haemocyte in the process of losing its structured compactness. 12. A haemocyte with ruptured boundary and disorganised organelles, (m, mitochondria; er, endoplasmic reticulum). (Magnifications: 1 and 4 × 3,000; 2, 3, 5 and 6, × 6,000; 7 and 8, × 2,000; 9, × 4,000; 10, × 800; 11, × 58,000; 12, × 10,000).

even in this cell the plasma membrane is punctured or dissolved because of extreme fragility (figure 6).

In addition to topographical changes, the internal organisation of cells is disturbed beginning with an intensive vacuolization of cytoplasm (figure 10) to be followed by loss of compactness of structured granules (figure 11) and finally totally disintegrated cells containing organelles (e.g. mitochondria and endoplasmic reticulum) bound by disrupted membrane, are left. Later, because of thinning of



Figures 1-12.

plasma membrane there remains no restraint on these organelles and they ultimately pass out of the cell (figure 12).

4. Discussion

The occurrence of haemocytic reactions to foreign bodies or microorganisms has been studied in many insects and phagocytosis or encapsulation or nodule formation etc. are usually the resultant phenomena (Francois 1975; Ratcliffe and Rowley 1979; Wago and Ichikawa 1979; Ratcliffe and Walters 1983; Walters and Ratcliffe 1983; Wago and Kitano 1985). In *Pieris rapae crucivora* and *Bombyx mori* scanning electron microscopical studies have shown that plasmatocytes or granular haemocytes are active participants in such processes by spontaneously extending their lamellipods or filopods in recognition of foreignness (Wago 1980, 1983; Wago and Kitano 1985). Similar observations are true for *Spodoptera litura* where filopods entrap ruptured cells (Saxena *et al* 1988). The effect of many insecticides and biological agents has been studied on insect haemocytes but majority of such details pertain either to light microscopy or transmission electron microscopy (Zaidi and Khan 1977; Feir 1979; Ratcliffe and Rowley 1979). Since an analysis of the effect of any phytochemical on surface morphology of haemocytes is needed, the present investigations on *Dysdercus* haemocytes by the topical application of plumbagin are important. The difference between the blood of an untreated and treated bug was obvious by almost a complete absence of the elongation processes i.e. filopods in affected granular haemocytes as well as plasmatocytes. These two types of cells normally provide protection against any invading microorganism and the suppression of their cytoplasmic processes indicates clearly their inability to cope with the situation created in the body by the compound. Also, the plasma membrane of cells becomes fragile and this fragility leads to punctures (as in oenocytoid) or dissolution which makes the outer membrane incapable of keeping a hold over the cytoplasm whose gradual oozing leaves behind a cell having a skeletal interconnection of cytoplasmic strands (as in granular haemocyte). The abundance of fat droplets shields the adipohaemocyte but, as compared to a normal healthy cell, it presents a shrivelled up appearance in the scanning micrograph.

The transmission microscopy used as a sequel to scanning electron microscopy confirmed the cells disintegration which, starting with vacuolization and loss of compactness of organelles, ends with an emptied cytoplasm which is devoid of its components and covered by a ruptured membrane. Such a haemocyte is ultimately lost from the haemolymph—a fact corroborated by differential and total counts. Shapiro (1979) has dealt at length with the changes in haemocyte populations in response to particulate and non-particulate materials, poisons and diseases. In *Galleria mellonella* injection of the bacterium *Bacillus cereus* into haemocoel rapidly depletes the number of circulating plasmatocytes (Chain and Anderson 1983). An alteration in haemocyte counts is induced upon injection of yeast cells in *Poeciloceris pictus* haemocoel (Sharma *et al* 1986). Unlike all this injected foreign matter, plumbagin is a plant product whose topical application to the body of *D. koenigii* causes a rapid decline of almost all the cell types. The prohaemocytes are totally eliminated from the blood while granular haemocytes and plasmatocytes decline continuously from 24–48 h of treatment. The fall in counts of these major

representative types of haemocytes in treated insects increases the percentage of oenocytoids and adipohaemocytes in DHC (table 1). However the THC clearly project the overall total reduction in the counts in comparison with those of the controls.

With the present work, it is difficult to state conclusively the reason for changes in haemocyte morphologies and the steep decline in their numbers. The question whether the cells are affected directly or via some physiological or endocrinological pathway is yet to be answered in spite of the report that in *D. cingulatus* retardation of growth by plumbagin is on account of its effect on endocrine system (Joshi *et al* 1988).

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Changes in nucleic acids and protein content in relation to body size in the prawn *Penaeus indicus* H Milne Edwards

GEORGE THOMAS and A D DIWAN

Central Marine Fisheries Research Institute, Cochin 682 031, India

MS received 17 July 1989; revised 5 February 1990

Abstract. This study was carried out to find out the relationships of RNA, DNA content, RNA/DNA ratio and protein levels with different size length individuals of *Penaeus indicus*. RNA, DNA content, and RNA/DNA ratio were found to be high in small sized prawns, with increased amount of protein. There was a decline in RNA concentration as size of the prawns increased, thus showing an inverse relationship. Though DNA values also showed a slight decline with increase in size, among large sized prawns there was a tendency to conserve DNA content. RNA/DNA ratio has been found useful in correlating growth (in terms of protein increase) only in small sized prawns.

Keywords. Body size; nucleic acids; protein content; *Penaeus indicus*.

1. Introduction

Growth in animals has traditionally been expressed as an increase in either length or live weight or dry weight body mass (Buckley 1981). In recent times, a new tool has been introduced to determine the growth rate especially during the exponential phase of the growth of animals by measuring the RNA/DNA ratio in the cell or tissue (Bulow 1970; Buckley 1981, 1984). Considerable work has been carried out to correlate the concentration of RNA to growth rate in a number of marine organisms (Sutcliffe 1965; Pease 1968; Dagg and Littlepage 1972). Bulow (1970) described RNA-DNA ratio as indicators of growth rate in the fish *Notemigonus crysoleucas*. Buckley (1979a, b) found that the RNA/DNA ratio was an useful index of nutritional status in the larval Atlantic cod *Gadus morhua* and the winter flounder, *Pseudopleuronectes americanus*. Recent laboratory studies have also demonstrated positive relations between food availability and larval RNA and DNA ratio and between RNA/DNA ratio and growth rate of larval fish (Bulow 1970; Buckley *et al* 1984) and a juvenile crab, *Callinectes sapidus* (Wang and Stickle 1986). During the present study, an attempt has been made to find out correlations if any between RNA and DNA concentrations, RNA/DNA ratio and protein content in relation to different sized individuals of the penaeid prawn, *Penaeus indicus*.

2. Materials and methods

The penaeid prawns, *P. indicus* of different body lengths ranging from 24–147 mm were used. The prawns of smaller size ranging from 24–48 mm were collected from the nursery pools using scoop nets and the other size-groups up to the size of about 147 mm were collected from growout ponds of the Marine Prawn Hatchery Laboratory, Narakkal with the help of cast nets. The collected specimens were

transported to the laboratory either in transportation bags or 60 l capacity plastic bins as and when required for analysis. For the estimation of RNA, DNA and protein content, animals in the intermoult stage were isolated by the method of Diwan and Usha (1985). Generally the whole animal was used for analysis after removing the hard integument.

2.1 RNA and DNA protein

RNA was extracted as per the methods described by Schmidt and Thannhauser (1945) and further modified by Munro and Fleck (1966). Yeast RNA was used to prepare the standard curve. The DNA content of the sample was extracted by indole method (Ceriotti 1952). Highly polymerized calf thymus DNA was used to prepare the DNA standard curve. Protein was analysed by the Biuret method (Gornall *et al* 1949). Bovine serum albumin crystals were used to prepare the protein standards. Taking size as independent variable, polynomial regressions of the forms $Y = a + b_1x + b_2x^2$ were fitted where x referred to size, Y referred to RNA, DNA and protein respectively and a , b_1 and b_2 are constants determined by the method of least squares. All the coefficients were found to be significant at 5% level. Regression function was not fitted for RNA/DNA ratio since it is a derived value.

3. Results and discussion

Regression coefficients and their standard errors on the data obtained are given in table 1. The results of RNA, DNA content, RNA/DNA ratio and protein levels in relation to different size-length of animals are summarised in figures 1-4. The high level content of RNA, DNA and RNA/DNA ratio with increase in protein levels in smaller prawns of up to 42 mm recorded here, might be due to the faster growth in early phase of life cycle of the animal. Moreover, these animals were collected from nursery pools of the farm where artificial feeding was done regularly. From size 44 mm onwards, almost a decreasing trend for RNA values could be seen reaching a lowest value of $3.2 \mu\text{g mg}^{-1}$ for the 147 mm size (figure 1). Dagg and Littlepage (1972) found increased amount of RNA concentration during early phase

Table 1. Regression coefficients and their standard errors.

	Coefficients	SE(b)
Polynomial regression of RNA on size	$a = 15.3792$	
	$b_1 = -0.1921$	0.0313
	$b_2 = 0.00079$	0.00018
	$r^2 = 0.7245$	
Polynomial regression of DNA on size	$a = 1.1382$	
	$b_1 = -0.0085$	0.0021
	$b_2 = 0.000024$	0.000012
	$r^2 = 0.7627$	
Polynomial regression of protein on size	$a = 80.9909$	
	$b_1 = 2.0558$	0.0895
	$b_2 = -0.0064$	0.0005
	$r^2 = 0.9882$	

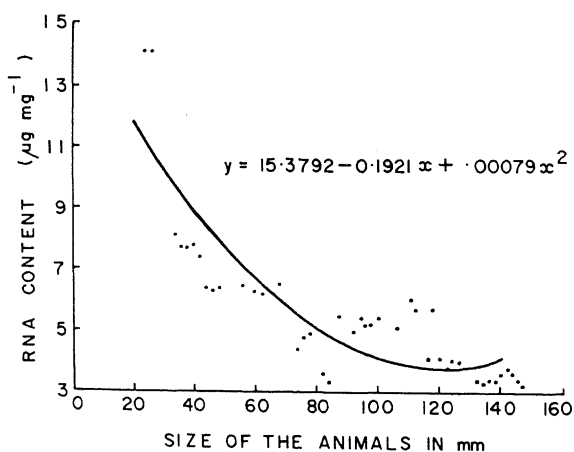


Figure 1. RNA content in different size lengths of *P. indicus*.

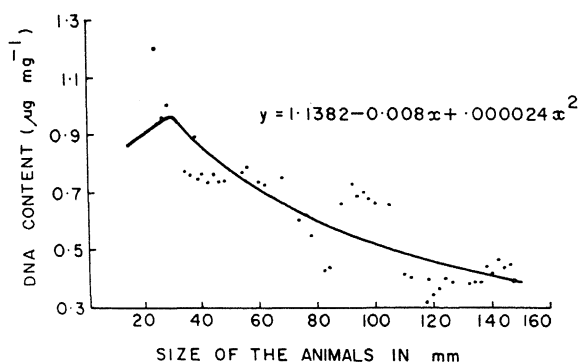


Figure 2. DNA content in different size lengths of *P. indicus*.

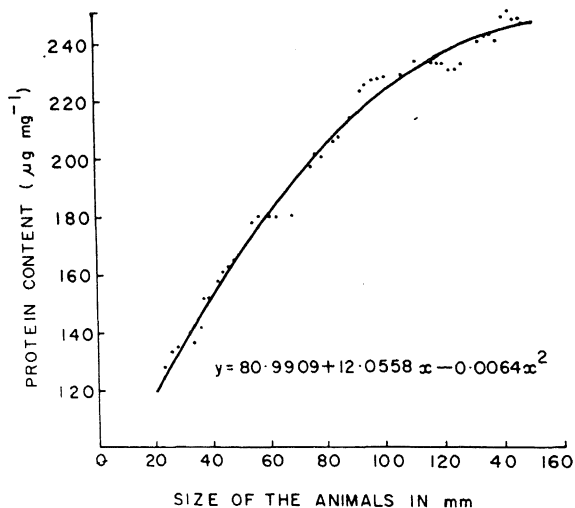


Figure 3. Protein content in different size lengths of *P. indicus*.

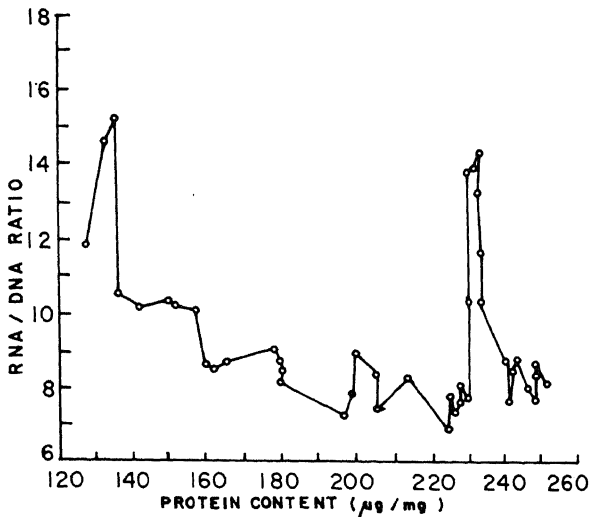


Figure 4. Variations in RNA/DNA ratio to protein content in different sized *P. indicus*.

of growth in *Artemia salina* but thereafter they did not find any significant change; they have also noticed the highest RNA concentration in youngest *Euchaeta elongata* and lowest in adults.

The highest DNA value was recorded for smallest-size *P. indicus* (24 mm) and thereafter up to the size of 106 mm, the values declined but remained steady. Sudden decline in the values were noticed from 110 mm size onwards but the values never reached lowest with increase in size like RNA content (figure 2). While estimating RNA/DNA ratio in relation to growth rate of a fish, Bulow (1970) found that there was slight decrease in DNA content with increased growth and slight increase with increased weight loss and further explained that this change was probably due to change in the cytoplasmic volume. While working on *A. salina* Dagg and Littlepage (1972) showed that the increased growth is represented by increase in protein and a decline in the DNA content but he was unable to predict the reason for such a decline. The present findings of decrease in DNA content with increase in size agrees with the observations of Bulow (1970) and Dagg and Littlepage (1972) but no inverse correlation was seen with increased protein level like RNA, as there was great variation in the DNA values. It could be noted that most of the times the animals maintained a general tendency to conserve DNA with increase in size.

Large amount of work has been carried out to correlate RNA/DNA ratios as a growth index. Bulow (1970) while working on fish 'golden shiners', demonstrated that RNA/DNA ratio gets affected due to starvation, thus reflecting growth index. Buckley (1981, 1984) in his works indicated the importance of RNA/DNA ratio and its correlation with protein growth rate especially in the phase of exponential growth. In the present work high ratio of RNA/DNA was observed up to 42 mm size, the highest was recorded for 28 mm size. The findings reported here for smaller prawns are similar to those observed for fish larvae by a number of workers (Bulow 1970; Buckley 1981, 1984). From 44 mm size onwards RNA/DNA ratios were slightly low but remained steady up to 106 mm and again for 132–147 mm sized

animals (figure 3). While the sudden rise in the values of ratio in the prawns of size 110–126 mm was interesting no proper reasons could be attributed. The only possible explanation is that, this high ratio could be a good indicator of the better nutritional status of the animals at the time of collection. Buckley (1984) while investigating RNA/DNA ratio values of cod, haddock and sand lance larvae, which were collected from varied locations found that, the majority of the larvae had high RNA/DNA ratio. Among the sand lance larvae 7% of the collected samples had low RNA/DNA ratio indicating that a substantial portion of sand lance collected were in poor condition, probably due to inadequate nutrition. It is stated that the high RNA/DNA ratios could indicate either healthy, well fed, rapidly growing fish, while those under stress have low ratios (Buckley *et al* 1984). In the present study prawns above 54 mm were collected from growout ponds of CMFRI laboratory. In such a situation as in Buckley's (1984) findings RNA/DNA ratios can be used as a good indicator of nutritional status of the animals. Irrespective of steadiness in the values of RNA/DNA ratio in large sized prawns, there was continuous increase in the protein content, thus showing no relationship between these two factors after attainment of a particular size (figure 4). In fact there is no information available at present about RNA/DNA ratio and its relation to protein content in adult sized animals.

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Biology of *Aphanogmus fijiensis* (Ferriere) (Hymenoptera: Cera- phronidae) a hyperparasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) through *Apanteles taragamae* Viereck (Hymenoptera: Braconidae)*

CLEMENT PETER and B V DAVID

Department of Entomology, Fredrick Institute of Plant Protection and Toxicology,
Padappai 601 301, India

MS received 25 October 1989

Abstract. Studies were conducted on the hyperparasite *Aphanogmus fijiensis* (Ferriere) (Hymenoptera: Ceraphronidae) to determine its biology through *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). *Aphanogmus fijiensis* is a gregarious ectoparasite and developed on the prepupal and pupal stages of *Apanteles taragamae* present inside the host cocoon. Mean development time from egg to adult was 12.28 days at $26.43 \pm 2.53^\circ\text{C}$. There is no preoviposition period; the host is paralysed before oviposition, freshly formed to 3-day old cocoons are preferred for oviposition and mean longevity for both sexes was 4.86 days.

Keywords. *Aphanogmus fijiensis*; hyperparasite; biology.

1. Introduction

Apanteles taragamae Viereck is a gregarious endoparasite and was recorded for the first time from *Diaphania indica* Saunders on snake gourd from Sri Lanka (Wilkinson 1931). In 1948, Bhatnagar listed 13 hosts of *A. taragamae*. During the studies on biological control of *D. indica* at Padappai, it was observed that *A. taragamae* was the major parasite of this insect pest. Even though parasitism of *D. indica* during certain months was as high as 80%, the pest was not completely controlled. It was observed that the cocoons of *A. taragamae* were attacked by the hyperparasite, *Aphanogmus fijiensis* (Ferriere). This species was originally described as *Calliceras fijiensis*, a hyperparasite of *Tirathaba* sp. through *Apanteles tirathabae* Vlk. (Ferriere 1933). It has also been recorded as a hyperparasite of *Microgaster urticornis* Granger (Williams 1951). Later it was reported as a parasite of *Cremastus* (*Trathala*) *flavo-orbitalis* (Cameron) in Fiji (Hinckley 1963). *Ceraphron* sp. near *fijiensis* was also recorded as a hyperparasite of the coconut pest, *Artona atoxantha* Hampson through *Apanteles artonae* Roh. from Malaya (Lever 1964). This is the first record of *A. fijiensis* as a hyperparasite of *D. indica* through *A. taragamae*. Hence, in the present investigation the biology of this hyperparasite was studied.

2. Materials and methods

To study the biology of the secondary parasite it was essential to maintain a culture

of the primary parasite. The following procedure was followed to rear *A. taragamae* in the laboratory.

2.1 Rearing of stock culture of primary parasite

Twenty-five third-instar *Diaphania* larvae were released on a bouquet of *Coccinia* leaves inserted into a glass vial (6×1.5 cm) and placed inside a plastic jar (12×10 cm). Three mated *A. taragamae* females were released into each jar. The larvae were exposed to the parasite for 12 h. At the end of this period the parasites were removed from the jar and the larvae allowed to feed on the leaves. The leaves were changed periodically until the parasite larvae completed their development inside the host larvae and the cocoons were formed. These cocoons were collected from the rearing jars and placed in specimen tubes.

2.2 Biology of hyperparasite

Freshly formed *A. taragamae* cocoons were exposed to *A. fijiensis* adults in glass specimen tubes for 1 h. After this the adults were separated from the cocoons and after 12 h the cocoons were dissected at periodic intervals of 6 h until hatching of the hyperparasite eggs was observed. After the incubation period, the cocoons were dissected every 24 h and the development of the larval stage was observed, measured and drawn. To establish the larval period the parasitized host cocoons were dissected at periodic intervals using a Carl Zeiss Zoom Citoval-2-Stereo-microscope. The parasite larvae were measured with a calibrated ocular micrometer and drawn using a camera lucida attached to a Carl Zeiss Laboval 4 Compound microscope. The prepupal and pupal periods were also recorded. The rearing was done at $26.43 \pm 2.53^{\circ}\text{C}$ and 65-80% RH.

The preoviposition and oviposition periods were determined by exposing freshly formed primary parasite cocoons to the hyperparasite at regular intervals of 24 h beginning with the day of emergence. The cocoons were dissected after each exposure and the number of parasitized cocoons as well as the number of eggs laid per cocoon counted. The host cocoons were exposed continuously until the female died. Adult longevity was determined by feeding honey solution to freshly emerged males and females. The age of the host cocoons preferred for oviposition was determined by exposing 0-5-day old *A. taragamae* cocoons to *A. fijiensis* adults for 12 h. After exposure the cocoons were placed in glass specimen tubes. The development time for each age group was recorded.

The stock culture of *A. fijiensis* was maintained in the insectary by rearing it on *A. taragamae* cocoons. The cocoons were exposed for 6 h after which the adults were separated from the cocoons. The exposed cocoons were placed in glass specimen tubes until the adults emerged.

3. Results and discussion

3.1 Immature stages

3.1a Egg: The egg is opaque, shining white with minute punctate markings on

the chorion. It is spindle shaped and pointed at one end and slightly rounded at the other end (figure 1A). The length averaged 0.19 and 0.05 mm wide (table 1).

1.1b Larva: The larva passes through 3 stages. The freshly hatched larva is white and translucent and remains attached to the spot where the egg was deposited (figure 1B,C). The head is rounded and the body has 12 segments. The length of the freshly hatched larva averages 0.26 mm and the width 0.125 mm. The second instar larva is very similar to the first instar but is larger and has 13 segments. The body is translucent, smooth and globular (figure 1D,E). The size of larvae of this instar averaged 0.56 mm in length and 0.30 mm in width. The third instar larva has a curved body, broad at the anterior region and tapering to the posterior. It is translucent white at first and then turns opaque. The head is almost embedded in the broad anterior region. The entire surface of the body is covered with small tubercles. These are absent on the last segment which is smooth and rounded (figure 1F). The length of full grown larva averaged 1.27 mm and width at the middle was 0.54 mm.

The mandible is simple, pointed and well sclerotized (figure 1H). At the beginning of the first instar it measured 0.02 mm in length and 0.012 mm in width at the base. The size increases to 0.05 mm in length and 0.03 mm in width at the end of the

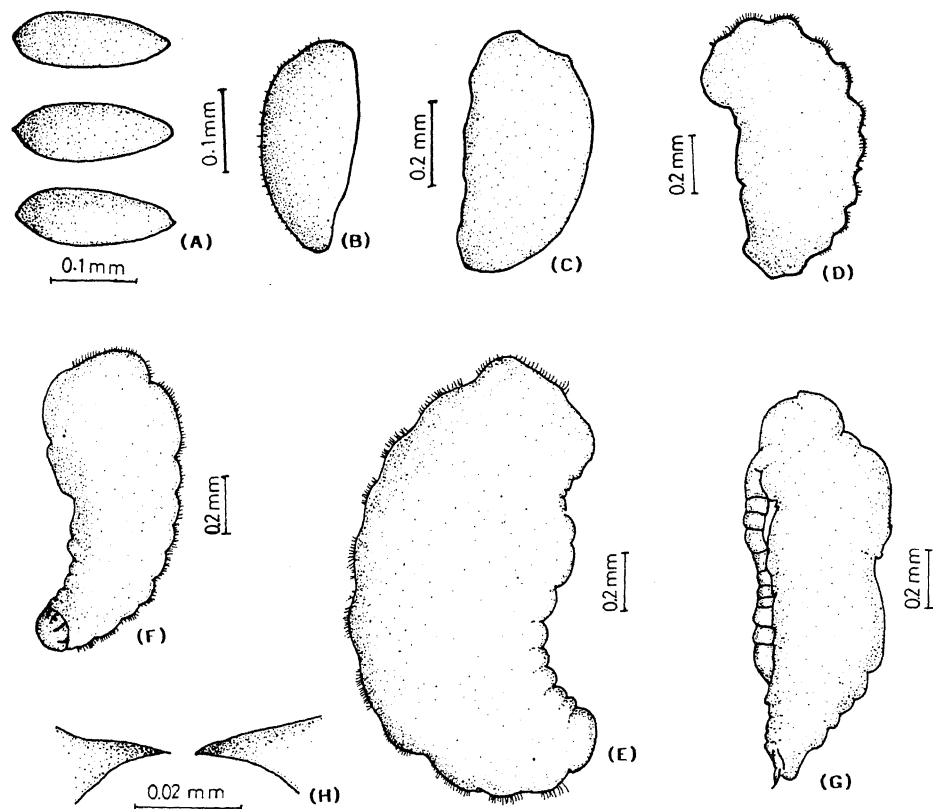


Figure 1. Developmental stages of *A. fijiensis*. A. Egg. B, C. I instar larva. D, E. II instar larva. F. III instar larva. G. Pupa. H. Mandibles.

Table 1. Mean size and duration of immature *A. fijiensis* reared at $26.43 \pm 2.53^\circ\text{C}$.

Stage	n	Length (mm)	Width (mm)	Duration (h)
		$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$	
Egg	25	0.19 ± 0.12	0.05 ± 0.02	24–36
I instar	20	0.26 ± 0.04	0.12 ± 0.04	1–2
II instar	18	0.56 ± 0.03	0.30 ± 0.04	2
III instar	15	1.27 ± 0.10	0.54 ± 0.03	1–2
Prepupa	12	1.17 ± 0.34	0.56 ± 0.27	1
Pupa	12	1.19 ± 0.27	0.45 ± 0.52	4–7

third instar. The shape of the mandibles is similar at all stages of larval development.

3.1c Pupa: The freshly formed pupa, bright white at first, gradually turns dirty white. The appendages such as antennae, legs and wing pads are pressed to the body (figure 1G). The length of the pupa averaged 1.19 mm and the width at the thoracic region averaged 0.45 mm.

3.2 Life history

A. fijiensis develops as gregarious ectoparasite on the pupa of *A. taragamae* inside the cocoon. The eggs are laid on the body of the host through the host cocoon. The egg period ranges from 24–36 h and the larva on hatching commences feeding at the point where it was laid. It continues to feed for 4–6 days. At the end of this period the host is entirely consumed and only the full grown larvae of the hyperparasite remain inside the host cocoon. The larvae discharge the meconium and enter the prepupal stage. The meconium which remains attached to the posterior end of the larva is smooth, spherical and dark brown. The prepupal stage lasts about a day after which it turns into pupa.

The pupal period ranges from 4–7 days. The total life cycle of *A. fijiensis* from egg to adult studied with 60 cocoon masses exposed to this hyperparasite averaged 12.28 days (range 11–15 days).

3.3 Adult

3.3a Oviposition: The females commence oviposition on the day of emergence. There is no preoviposition period and oviposition continue as long as the females live. The female has a long concealed ovipositor. During oviposition the ovipositor is extended at an acute angle to the body to probe the cocoon as if stinging it. Then the region behind the ovipositor enlarges and the egg is forced through the ovipositor. Oviposition lasts 30–60 s after which the female moves around rapidly and stops frequently to drum the cocoon with its antennae.

The eggs are laid on the body of the host which is generally in the prepupal stage. There is no specific site for oviposition but the eggs are distributed on different parts of the body. The number of eggs found in each cocoon averaged 6.30 (range 3–22 eggs/host) and out of these an average of 5.25 individuals (range 4–11) completed development.

3.3b *Host paralysis*: The ovipositing female paralyses the host in the prepupal stage. The prepupa in the unexposed cocoon develops into a pupa within one day, while the prepupa in the exposed cocoon remains in the same stage without further development. It is therefore apparent that before oviposition the female paralyses the host in the cocoon.

3.3c *Host age preferred for oviposition*: An experiment was conducted to determine the stage of the host preferred by *A. fijiensis* for oviposition. Cocoons of *A. taragamae* of different age groups (ranging from freshly formed to 4-day old) were exposed to adults of *A. fijiensis*. The study indicated that cocoons up to 3 days old were acceptable for oviposition and development.

In 4-day old cocoons the pupa of the host is fully developed and not suitable for the development of *A. fijiensis*. No hyperparasites emerged from 4-day old cocoons whereas adults of *A. taragamae* emerged after 1 to 2 days. The size of the hyperparasite adults emerging from 3-day old cocoons is smaller than those from 1 and 2-day old cocoons; in addition, the date of emergence of adults is extended by one day (i.e. 14 days for 3-day old cocoons). Similarly, the size of adults emerging from hosts parasitised as freshly formed cocoons is reduced and the date of emergence is delayed by one day.

3.3d *Longevity*: When kept in tubes and fed on 20% honey solution the adult life span averaged 4.86 days (maximum 9 days and minimum 2 days) for both sexes. Without food under similar conditions the adults survived only two days.

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Influence of biochemical parameters of host plants on the biology of *Dialeurodes vulgaris* Singh (Aleyrodidae: Homoptera)

R SUNDARARAJ and B V DAVID

Fredrick Institute of Plant Protection and Toxicology, Padappai 601 301, India

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Abstract. The influence of biochemical parameters of the host plants viz. *Jasminum multiflorum* Andr., *Jasminum sambac* Ait., *Jasminum grandiflorum* Linn., *Jasminum auriculatum* Vahl., *Jasminum pubescens* Willd. and *Jasminum flexile* Vahl. on the biology of *Dialeurodes vulgaris* was studied. The results indicate remarkable variation in the biochemical parameters of host plants which affected significantly the biology of the insect. The per cent survival and growth index have been considerably low in *Jasminum pubescens*, *Jasminum auriculatum* and *Jasminum grandiflorum* and the same trend has been noticed with regard to fecundity and longevity also. The most preferred hosts have been *Jasminum sambac* and *Jasminum multiflorum*. It is observed that high amount of phenol and excessive accumulation of sugars in host plants make them nonpreferred while high amount of amino acids makes the plants more preferred by *Dialeurodes vulgaris*.

Keywords. *Dialeurodes vulgaris*; jasmine; host preference.

1. Introduction

Several insects infest jasmine (*Jasminum sambac*) crop in south India (David 1958). Among them the jasmine whitefly *Dialeurodes vulgaris* Singh has been noticed to be endemic on the cultivars of jasmine in Tamil Nadu. There are 7 cultivars of jasmine belonging to 6 species of the genus *Jasminum* and all of them are infested by *D. vulgaris*. The present study reports on the quantitative levels of certain biochemical parameters of these cultivars and their influence on the biology of the whitefly on them.

2. Materials and methods

Seven cultivars of jasmine viz. Kundumalli (*J. multiflorum* Andr.), Maduramalli (*J. sambac* Ait.), Adukkumalli (*J. sambac* Ait.), Jathimalli (*J. grandiflorum* Linn.), Virijathi (*J. auriculatum* Vahl.), Kakkadamalli (*J. pubescens* Willd.) and Nithyamalli (*J. flexile* Vahl.) were raised in polythene bags with soils. Transparent plastic cages (8 × 6 cm) with screw cap at one end and the other end covered with muslin cloth were prepared. A part of the host plant was enclosed inside the cage. Whiteflies from the stock culture were collected using an aspirator and released into the cages for oviposition on the leaves. After 24 h the whiteflies and the cages were removed and observations recorded for incubation period, nymphal period, total developmental period, percentage survival, growth index, longevity of male and female and fecundity. There were 5 replications for each study.

Leaf samples of the host plants were simultaneously analysed for quantitative estimation of biochemical parameters. Leaf extracts were obtained using 80% ethanol. The reducing sugars were determined first. Subsequently the nonreducing sugars in the extract were hydrolysed to reducing sugars and the total sugars were

estimated by Nelson's (1944) method. By subtracting the reducing sugars from the total sugars, the nonreducing sugars were estimated and expressed as glucose equivalents. Free amino acids, protein and phenol were estimated following the methods, respectively of Moore and Steins (1948), Lowry *et al* (1951) and Bray and Thorpe (1954) (Outlined by Mahadevan and Sridhar 1982).

3. Results and discussion

The data presented in table 1 clearly indicate that the total developmental period have been considerably short whereas the per cent survival and growth index have been appreciably high in *J. sambac* and *J. multiflorum* followed by *J. flexile*, and the same trend has been noticed with regard to fecundity also. Hence the most preferred hosts have been *J. sambac* and *J. multiflorum* whereas *J. pubescens* proved to be the least preferred one.

3.1 Influence of biochemical parameters of host plants on *D. vulgaris*

The data on reducing sugar, non reducing sugar, total sugar, free amino acids, protein and phenol for the different species of *Jasminum* are given in table 2. The most unfavourable host *J. pubescens* (Kakkadamalli) contains the highest amount of phenol (21.238 mg/g) followed by *J. grandiflorum* (Jathimalli) which contains 9,000 mg/g. When phenol is sufficiently synthesised it combines with the sugars obtained as primary product of photosynthesis to form tannins. The tannins combine irreversibly with the available protein and form the indigestible complexes which result in the nutritional deficiency in the host plants which in turn affects the percentage of infestation and host preference (Raman and Ananthakrishnan 1986). On the contrary *J. auriculatum* (Virijathi) recorded lowest amount of phenol

Table 1. Influence of hosts on the developmental biology of *D. vulgaris*.

Host	Incubation period (days)	Nymphal period (days)	Total developmental period (days)	Survival (%)	Growth index	Longevity (days)		Fecundity
						Male	Female	
Kundumalli (<i>J. multiflorum</i>)	8.06	24.68	32.74	71.26	2.18	11.10	12.93	82.00
Maduramalli (<i>J. sambac</i>)	8.13	24.45	32.58	68.78	2.11	10.57	12.10	75.33
Adukkumalli (<i>J. sambac</i>)	8.26	24.53	32.79	72.17	2.20	9.77	11.47	71.67
Jathimalli (<i>J. grandiflorum</i>)	8.32	26.32	34.63	54.26	1.57	6.63	7.40	46.63
Virijathi (<i>J. auriculatum</i>)	8.62	27.00	35.63	47.91	1.36	6.26	6.57	36.00
Kakkadamalli (<i>J. pubescens</i>)	8.38	26.60	34.97	39.26	1.12	4.57	5.63	26.33
Nithyamalli (<i>J. flexile</i>)	8.84	24.40	33.24	63.83	1.91	9.83	10.13	68.67
CD ($P = 0.05$)	NS	1.46	1.15	5.71	0.20	1.48	1.45	10.07

Table 2. Biochemical parameters* of host plants of *D. vulgaris*.

Host	Reducing sugar	Non reducing sugar	Total sugar	Free amino acids	Protein	Phenol
Kundumalli (<i>J. multiflorum</i>)	32.565	23.495	55.727	2.512	22.248	4.694
Maduramalli (<i>J. sambac</i>)	33.689	25.869	58.557	2.776	19.748	6.492
Adukkumalli (<i>J. sambac</i>)	27.963	20.341	48.304	2.530	26.163	4.805
Jathimalli (<i>J. grandiflorum</i>)	22.141	10.315	32.456	2.319	22.443	9.000
Virijathi (<i>J. auriculatum</i>)	18.324	5.547	23.871	1.886	13.336	1.143
Kakkadamalli (<i>J. pubescens</i>)	48.383	7.721	56.103	2.093	23.808	21.238
Nithyamalli (<i>J. flexile</i>)	68.643	9.641	73.285	2.606	26.489	3.139
CD ($P=0.05$)	5.616	11.110	10.724	0.148	3.063	0.678

*Values in mg/g (mean of 3 replications).

(1.143 mg/g) and its non preference may be due to the low nutritive contents viz. reducing sugar (18.324 mg/g), non reducing sugar (5.547 mg/g), total soluble sugar (23.871 mg/g), free amino acids (1.886 mg/g) and protein (13.336 mg/g). House (1969) stated that in general all the phytophagous insects have quite similar qualitative nutritional requirements but it is the quantitative factor that plays a more decisive role in insect host plant relationship. The total soluble sugar was found to be the highest (73.285 mg/g) in *J. flexile* (Nithyamalli) which is moderately preferred. The antibiosis or non preference of the host plants was correlated with the excessive accumulation of total sugars (Ananthakrishnan 1986). The favourable hosts viz. *J. sambac* (Adukkumalli/Maduramalli) and *J. multiflorum* (Kundumalli) contain moderate amount of sugars and protein and low amount of phenol when compared with *J. pubescens* (Kakkadamalli) and *J. grandiflorum* (Jathimalli). The amount of free amino acids was higher in preferred hosts being 2.512 mg/g in *J. multiflorum* (Kundumalli), 2.776 mg/g in *J. sambac* (Maduramalli) and 2.530 mg/g in *J. sambac* (Adukkumalli) which is in agreement with the findings of David and Paul (1973). However, *J. flexile* (Nithyamalli) recorded higher amount of amino acid and its non preference may be due to excessive accumulation of sugars (73.285 mg/g).

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On reconfirming the evidence for pre-imaginal caste bias in a primitively eusocial wasp

RAGHAVENDRA GADAGKAR, SEETHA BHAGAVAN,
RASHMI MALPE and C VINUTHA

Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India

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Abstract. Caste is usually thought to be determined entirely in the adult stage in most primitively eusocial wasps and bees. A pre-imaginal caste bias has however been recently discovered in the primitively eusocial wasp *Ropalidia marginata*. This study also suggested that reigning queens and possibly other adults may influence the production of new queens and implied at least partial support to the parental manipulation or sub-fertility hypothesis for the evolution of insect sociality. The interest of these results prompted an attempt at their reconfirmation. Complete reconfirmation has now been obtained using data from an independent experiment and two additional methods of data analysis. We therefore conclude that caste is at least partly determined prior to eclosion in the primitively eusocial wasp *Ropalidia marginata* which lacks morphological differentiation between egg-layers and non-egg-layers.

Keywords. *Ropalidia marginata*; social wasps; caste determination; evolution of sociality.

1. Introduction

Evolution of altruistic behaviour is one of the prime concerns of sociobiology. Eusocial insects such as ants, bees and wasps, which are characterized by overlap of generations, co-operative brood care and reproductive caste differentiation, show the most extreme forms of altruism and hence are often favoured as model systems in sociobiology. Of these, primitively eusocial insects such as many species of bees and wasps lack morphological differences between reproductive and non-reproductive castes and thus show considerable flexibility in the social roles that individuals may adopt. For this reason, such species are particularly suited for asking questions concerning the forces that mould the evolution of worker behaviour.

Most studies on these species however have assumed that caste is entirely determined in the adult stage. To verify this assumption, an experiment was set up to test the null hypothesis that 'all eclosing females in the primitively eusocial wasp, *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae) are potentially capable of laying eggs'. Of the 197 freshly eclosed females tested in this experiment, only about 50% built nests and laid eggs, although all animals were isolated from conspecifics and were provided with *ad libitum* food since eclosion. This result therefore permitted rejection of the null hypothesis and suggested that caste is at least partly determined prior to eclosion. In addition, the number of empty cells on the nest from which an animal ecloses and its own rate of feeding during adult life were found to be good predictors of the probability that it would become an egg-layer. The presence of empty cells may be considered an indication of the queen's declining condition. These findings were therefore interpreted to mean that

potential workers are produced when the queen of a colony is young and healthy or has a high influence on her colony for other reasons. Conversely, potential egg-layers were thought to be produced when the queen is old and unhealthy or her influence is otherwise low (Gadagkar *et al* 1988).

Because of the interest of these results and certain features of our methodology, we have considered it worthwhile to repeat the experiment. Here a complete reconfirmation of all our previous results using data from a new experiment and employing two additional methods of data analysis is reported.

2. Materials and methods

2.1 Experimental procedures

All experiments were performed using the primitively eusocial wasp *R. marginata* whose biology and social organisation have been described in some detail (Gadagkar 1980, 1985, 1990; Gadagkar *et al* 1982; Gadagkar and Joshi 1983). Naturally occurring nests were collected from around Bangalore (13°00' N and 77°32' E), cleared of adults and maintained in the laboratory. Females eclosing from these nests were isolated into individual 22 × 11 × 11 cm ventilated plastic jars. The animals so isolated were provided with a piece of soft wood as a source of building material and were provided an *ad libitum* diet of final instar *Corcyra cephalonica* larvae, honey and tap water from the same source. Records were maintained of the number of *C. cephalonica* larvae consumed by each animal throughout the experiment. The animals were observed for signs of nest-building and egg laying every day. Our experimental procedures are described elsewhere in more detail (Gadagkar *et al* 1988).

2.2 Data analysis

2.2a Logistic regression analysis: Each animal in our experiment was classified either as an egg-layer or a non-egg-layer. This classification thus yields a binary variable (which shall henceforth be called the dependent variable). We have therefore used the method of logistic regression analysis (Shanubhogue and Gore 1987) rather than simple linear regression analysis to analyse two models. In each model, several variables (which shall be referred to as independent variables) associated with each animal such as the number of eggs, larvae, etc. on its parental nest, its own feeding rate during adult life and indices of its body size were modelled to influence the probability of egg laying by females such that:

$$\ln [p/(1-p)] = \beta_0 + \sum \beta_i X_i.$$

This can be rewritten as

$$p = e^{\beta_0 + \sum \beta_i X_i} / 1 + e^{\beta_0 + \sum \beta_i X_i}.$$

Values of β_0 and β_i associated with each independent variable were obtained using the maximum likelihood criterion:

$$L = \left(\prod_{i=1}^m p_i \right) \left(\prod_{i=m+1}^n [1-p_i] \right),$$

where p is the probability of becoming an egg-layer, $1-p$ the probability of becoming a non-egg-layer, β_0 the intercept, β_i the regression coefficients, X_i the independent variables, L the likelihood, animals 1 to m the egg-layers, and animals $m+1$ to n the non-egg-layers.

2.2b *Comparison of means*: Mean values of independent variables for egg-layers were compared with those for non-egg-layers using a t test. Because some of the independent variables may not be normally distributed, we have also compared means using the non-parametric Mann-Whitney U -test.

2.2c *Correlation analysis*: Although the dependent variable is binary in nature, a corresponding variable which is not binary is the proportion of egg-layers among the animals eclosing from each nest. The association of this variable with those independent variables concerning the nests such as number of eggs, larvae, etc. was investigated by computing Kendall's rank correlation coefficient.

3. Results

3.1 *Need for reconfirmation*

The experiment described in Gadagkar *et al* (1988) showed that about half the animals tested became egg-layers and the other half non-egg-layers. The correlates of becoming an egg-layer or a non-egg-layer were investigated by performing what is sometimes known as a 'fishing expedition'. In other words, we had no specific hypothesis about which variables may be influencing the probability of an animal becoming an egg-layer. All variables for which data were available were tested and 2 out of 15 so tested were found to be significantly correlated with an animal's probability of becoming an egg-layer. Therefore the confidence one may entertain in such a result is perhaps limited. On the other hand, the results obtained by Gadagkar *et al* (1988) were novel and somewhat unexpected. Besides, they led to the discovery of the phenomenon of pre-imaginal caste bias in a primitively eusocial insect which lacks morphological caste differentiation. Although the extent of pre-imaginal caste bias seen cannot by itself account for eusociality in this species, these results imply at least some support for the parental manipulation (Alexander 1974) or sub-fertility (West-Eberhard 1975) hypothesis for the evolution of eusociality. All these factors prompted us to seek a reconfirmation of these results.

3.2 *Two methods of reconfirmation*

We have attempted to reconfirm the results of Gadagkar *et al* (1988) by the combination of two methods. First the experiment was repeated using a new set of animals from a new set of nests. Secondly, the possible involvement of the same variables was studied using two additional statistical techniques, viz. (i) comparison of mean values of independent variables for egg-layers and those for non-egg-layers and (ii) estimating correlation coefficients between the independent variables and the proportion of egg-layers among the animals eclosing from each nest. These two techniques and the original technique of logistic regression analysis have now been

applied to data from each of the two experiments as well as for data pooled from the two experiments.

3.3 *Strategies to deal with results from different statistical tests and different experiments*

One obvious problem that would arise when the same variables are studied by different statistical techniques or by using data from independent experiments is that the results may not be identical. The following strategy was used to deal with such potentially confirming or conflicting results.

When conflicting results were obtained within the same experiment but between different statistical tests, the variables involved were treated with suspicion and only those variables which were shown to be significant by all statistical tests were considered important.

Our strategy to deal with results from replicate experiments has been somewhat different. First, a new experiment was performed under conditions identical to those in the first experiment. Secondly, data pooled from both experiments were reanalysed. This gave us 3 sets of results namely those from experiment 1, from experiment 2 and from data pooled from experiments 1 and 2. Only those variables which were significant in the pooled data as well as in at least one of the individual experiments were accepted as being important in influencing the outcome of the experiment.

3.4 *Logistic regression analysis*

Results of both models using logistic regression are in table 1 and those pertaining to experiment 1 are from Gadagkar *et al* (1988). In experiment 1, three variables, viz. number of empty cells on parental nest, an animal's feeding rate during its adult life and its ocello-ocular distance (an index of body size) had coefficients which were significantly different from zero ($P < 0.05$). In the second experiment, only feeding rate has a regression coefficient which is significantly different from zero ($P < 0.05$). When data pooled from the two experiments are analysed, 3 variables, viz. number of pupae, number of empty cells and feeding rate are found to have coefficients significantly different from zero ($P < 0.05$). Using the strategy outlined in the previous section, we consider the roles of number of empty cells and feeding rate as having been reconfirmed. This strategy requires that the two other variables, viz. ocello-ocular distance and number of pupae not be considered important at this stage. It might be mentioned here that similar results are obtained if the proportion of empty cells is considered instead of the absolute number of empty cells.

3.5 *Comparison of means*

The mean values of each variable for egg-layers were compared with those for non-egg-layers as another method of detecting the correlates of egg laying. Our conclusions arising from such comparisons using a parametric *t*-test (shown in table 2) are similar to those from logistic regression analysis. Once again, the roles

Table 1. Results of logistic regression analysis.

Variables	Data from experiment 1			Data from experiment 2			Data pooled from experiments 1 and 2		
	Estimated coefficient (β)	Standard error	Z	Estimated coefficient (β)	Standard error	Z	Estimated coefficient (β)	Standard error	Z
Model 1: Nest properties as determinants of the probability of egg laying by eclosing females									
No. of eggs	-0.0071	0.0154	-0.4597	0.1610	1.0874	0.1480	-0.0031	0.0087	0.3550
No. of larvae	0.0043	0.0149	0.2869	0.1486	0.9882	0.1504	-0.0111	0.0101	-1.0976
No. of pupae	0.0155	0.0226	0.6841	1.0208	1.3245	0.7707	0.0305	0.0148	2.0518*
No. of parasitized cells	-0.1347	0.1442	-0.9340	10.9547	10.4664	1.0466	0.0368	0.0639	0.5762
No. of empty cells	0.0525	0.0217	2.4169*	0.3039	3.0698	0.0990	0.0401	0.0126	3.1943*
No. of males	0.1532	0.1736	0.8827	2.2228	56.1278	0.0396	0.0483	0.0813	0.5943
No. of females	-0.0158	0.0195	-0.8131	-1.6864	1.7881	-0.9431	-0.0268	0.0137	-1.9588*
Model 2: Feeding rate and body size as determinants of the probability of egg laying									
Feeding rate	5.9180	1.4919	3.9666**	9.3788	3.7503	2.5008*	6.0943	1.3484	4.5197**
Inter-ocular distance	-1.8912	7.2793	-0.2598	-1.8777	10.7721	-0.1743	-4.4015	5.5478	-0.7934
Ocello-ocular distance	-12.7920	6.0868	-2.1016*	2.8892	7.5265	0.3839	-8.4351	4.5252	-1.8640
Head width	1.3801	1.4764	0.9348	-1.5973	2.8099	-0.5685	0.4546	1.1975	0.3796
Head length	1.4794	1.4561	1.0160	-1.9246	2.0181	-0.9537	0.3137	1.0554	0.2972
Mesoscutum width††	-1.2484	1.5386	-0.8114	1.1383	1.8972	0.6000	-0.2781	1.1218	-0.2479
Mesoscutum length††	1.3517	1.3390	1.0095	0.7408	2.1653	0.3421	0.8050	1.0595	0.7598
Wing length	-0.2133	0.3223	-0.6618	0.5895	0.3727	1.5817	0.0482	0.2200	0.2189

†Based on the strategies described in section 3.3.

*0.05 $\geq P > 0.001$; ** $P < 0.001$.††This was erroneously labelled as mesoscutellum in Gadagkar *et al* (1988).

Table 2. Results of parametric comparison of means.

Variables	Data from experiment 1			Data from experiment 2			Data pooled from experiments 1 and 2		
	Mean \pm SE (egg-layers)	Mean \pm SE (non-egg- layers)	Z	Mean \pm SE (egg-layers)	Mean \pm SE (non-egg- layers)	Z	Mean \pm SE (egg-layers)	Mean \pm SE (non-egg- layers)	Z
No. of eggs	85.6970 \pm 5.5302	85.2755 \pm 5.9025	0.0521	93.1522 \pm 9.7799	118.2105 \pm 10.9205	-1.7093	88.0621 \pm 4.8761	94.4779 \pm 5.3655	-0.8849
No. of larvae	68.9798 \pm 5.2515	74.0204 \pm 5.6169	-0.6555	71.7174 \pm 8.4424	104.8158 \pm 10.5772	-2.4457*	69.8483 \pm 4.4600	82.6250 \pm 5.1300	-1.8796
No. of pupae	41.4747 \pm 3.7161	43.8673 \pm 4.0792	-0.4336	59.0870 \pm 5.4363	72.7632 \pm 7.3868	-1.4911	47.0621 \pm 3.1326	51.9412 \pm 3.7469	-0.9990
No. of parasitized cells	1.3434 \pm 0.2922	1.5306 \pm 0.3311	-0.4238	2.3843 \pm 0.5577	0.6579 \pm 0.2566	2.6822**	1.6483 \pm 0.2681	1.2868 \pm 0.2509	0.9845
No. of empty cells	10.2929 \pm 1.3680	6.2959 \pm 0.8551	2.4776*	23.9783 \pm 4.1328	8.4737 \pm 2.5568	3.1904***	14.6345 \pm 1.6866	6.9044 \pm 0.9414	4.0021***
No. of males	1.0808 \pm 0.2650	0.9082 \pm 0.2271	0.4947	2.6522 \pm 0.4798	0.2632 \pm 0.1494	4.7546***	1.5793 \pm 0.2432	0.7279 \pm 0.1704	2.8669***
No. of females	41.8384 \pm 3.3054	43.6531 \pm 3.5963	-0.3715	54.6522 \pm 4.3480	69.6316 \pm 5.8058	-2.0651*	45.9035 \pm 2.6829	50.9118 \pm 3.2069	-1.1978
Feeding rate	0.2240 \pm 0.0132	0.1485 \pm 0.0107	4.4522***	0.1606 \pm 0.0089	0.1259 \pm 0.0088	2.7685**	0.2019 \pm 0.0094	0.1410 \pm 0.0077	4.9948***
Inter-ocular distance	0.3134 \pm 0.0024	0.3183 \pm 0.0023	-1.4433	0.3187 \pm 0.0043	0.3190 \pm 0.0037	-0.0529	0.3153 \pm 0.0022	0.3185 \pm 0.0020	-1.1037
Ocello-ocular distance	0.5164 \pm 0.0025	0.5240 \pm 0.0032	-1.8485	0.5125 \pm 0.0049	0.5118 \pm 0.0030	0.1071	0.5150 \pm 0.0024	0.5199 \pm 0.0024	-1.1535
Head width	3.1981 \pm 0.0121	3.1923 \pm 0.0159	0.2867	3.1896 \pm 0.0208	3.2000 \pm 0.0187	-0.3708	3.1951 \pm 0.0107	3.1949 \pm 0.0123	0.0144
Head length	2.8237 \pm 0.0135	2.8188 \pm 0.0151	0.2453	2.8349 \pm 0.0227	2.8510 \pm 0.0243	-0.4849	2.8276 \pm 0.0118	2.8295 \pm 0.0129	-0.1082
Mesoscutum width	2.2478 \pm 0.0146	2.2620 \pm 0.0164	-0.6498	2.2255 \pm 0.0217	2.2088 \pm 0.0237	0.5203	2.2400 \pm 0.0121	2.2443 \pm 0.0136	-0.2352
Mesoscutum length	2.3422 \pm 0.0175	2.3424 \pm 0.0188	-0.0088	2.3049 \pm 0.0250	2.2861 \pm 0.0255	0.5257	2.3292 \pm 0.0144	2.3237 \pm 0.0153	0.2641
Wing length	9.9697 \pm 0.0667	10.0867 \pm 0.0742	-1.1730	9.8792 \pm 0.0900	9.6592 \pm 0.1227	1.4460	9.9382 \pm 0.0536	9.9442 \pm 0.0661	-0.0713

†Based on the strategies described in section 3.3.

*0.05 > P > 0.01; **0.01 > P > 0.005; ***P < 0.005.

of number of empty cells and feeding rate are reconfirmed. Because some of the variables may not be normally distributed, a non-parametric comparison of means using the Mann-Whitney *U*-test was also performed. These results (shown in table 3) permitted the same conclusions as the logistic regression analysis and parametric comparison of means. In experiment 2, the mean values of the number of females on the parental nest were significantly different between egg-layers and non-egg-layers but such a result was not obtained either in experiment 1 or in the pooled data. In experiment 2 and in the pooled data, the number of males on the parental nest is significantly different between egg-layers and non-egg-layers but this variable is not considered important because it did not have a coefficient significantly different from zero in the logistic regression analysis.

3.6 Correlation analysis

As far as variables associated with the parental nest (such as number of eggs, larvae, etc.) are concerned, there is yet another way in which the association of such variables with egg laying and non-egg laying by the experimental animals can be investigated. This is to compute the proportion of egg-layers among animals eclosing from each nest and calculate Kendall's rank correlation coefficients between this quantity and each variable. Such an analysis shows that number of empty cells is significantly correlated with the proportion of egg-layers eclosing from each nest (table 4). Three other variables, viz. the number of pupae, number of

Table 3. Results of non-parametric comparison of means.

Variables	Data from experiment 1		Data from experiment 2		Data pooled from experiments 1 and 2		Remarks [†]
	<i>U</i>	<i>Z</i>	<i>U</i>	<i>Z</i>	<i>U</i>	<i>Z</i>	
No. of eggs	4790.50	0.1522	690.50	-1.6998	9387.00	-0.7018	
No. of larvae	4661.50	-0.4767	630.50	-2.2583*	8804.50	-1.5561	
No. of pupae	4850.50	-0.0013	780.50	-0.8662	9756.00	-0.1543	
No. of parasitized cells	4767.00	-0.2599	588.50	2.9719***	9031.50	1.4725	
No. of empty cells	3838.50	2.5527*	521.00	3.3136***	7164.00	4.0115***	Accept
No. of males	4782.50	-0.2276	520.00	3.8954***	8711.50	2.1805*	
No. of females	4833.50	-0.0440	623.50	-2.3224*	9330.00	-0.7865	
Feeding rate	3213.50	4.0970***	898.00	2.6879**	7579.00	4.8130***	Accept
Inter-ocular distance	4283.00	-1.5339	1286.00	0.0882	10344.00	-1.1780	
Ocello-ocular distance	4068.50	-2.0626*	1145.00	1.1267	10345.00	-1.1768	
Head width	4657.50	-0.4876	1241.50	-0.3849	10728.50	-0.5980	
Head length	4652.50	-0.4999	1201.00	-0.6583	10556.50	-0.8289	
Mesoscutum width	4421.50	-1.0807	1295.00	0.0236	10501.50	-0.9023	
Mesoscutum length	4730.50	-0.3023	1277.50	0.1411	11058.50	-0.1523	
Wing length	4280.50	-1.4290	1142.50	1.0485	10749.50	-0.5665	

[†]Based on the strategies described in section 3.3.

*0.05 > *P* > 0.01; **0.01 > *P* > 0.005; ****P* < 0.005.

Table 4. Kendall's rank correlation coefficients between proportion of egg-layers eclosing from each nest and other variables associated with the nests.

Variables	Data from experiment 1		Data from experiment 2		Data pooled from experiments 1 and 2		Remarks [†]
	τ	Z	τ	Z	τ	Z	
No. of eggs	0.0220	0.1435	0.1633	0.9772	0.1466	1.3139	
No. of larvae	0.0220	0.1435	0.0995	0.5951	0.0963	0.8632	
No. of pupae	0.1733	1.1290	0.3080	1.8425	0.3087	2.7673**	
No. of parasitized cells	-0.0702	-0.4575	0.4739	2.8351***	0.2515	2.2545*	
No. of empty cells	0.2622	1.7081	0.3580	2.1416*	0.3231	2.8966***	Accept
No. of males	-0.1283	-0.8359	0.4561	2.7286**	0.2583	2.3154*	
No. of females	0.1470	0.9575	0.0066	0.0396	0.1784	1.5994	

[†]Based on the strategies described in section 3.3.

*0.05 > P > 0.01; **0.01 ≥ P > 0.005; ***P ≤ 0.005.

parasitized cells and number of males are also significantly correlated but by the criteria laid down in section 3.3, we do not accept these variables as being important at this stage.

Figure 1A shows the proportion of egg-layers eclosing from each nest as a function of the number of empty cells in that nest. Although, the number of empty cells is statistically significantly correlated with the proportion of egg-layers (table 4, row 5), there is much scatter in the data, preventing us from discerning any simple relationship between the two variables. A major source of error in this data must arise on account of our attempt to compute proportion of egg-layers for nests from which a very small number of animals were tested (in several cases only one or two animals were tested). For this reason, we have deleted all nests from which 6 or fewer animals were tested. Now our estimates of proportion of egg-layers will have less error and the nature of the relationship between proportion of egg-layers and empty cells should be easier to unravel. Figure 1B shows that animals have a small probability (~0.28) of becoming egg-layers even when the number of empty cells is zero and that the probability of becoming an egg-layer rises rapidly to about 0.6 when the number of empty cells is 20 and later rises gradually to about 0.9 when the number of empty cells reaches about 80.

4. Discussion

Gadagkar *et al* (1988) demonstrated a pre-imaginal bias in the caste of female *R. marginata* by showing that only 97 out of 197 females tested under laboratory conditions initiated nests and laid eggs while the remaining died without doing so. The results presented here reconfirm a substantial pre-imaginal caste bias because in a new experiment with 102 animals, only 53 laid eggs and the remaining 49 died without doing so. Gadagkar *et al* (1988) also concluded that the number of empty cells on the parental nest and an animal's feeding rate during its adult life are useful in predicting whether an animal will become an egg-layer or a non-egg-layer. The role of these variables was not postulated beforehand but, as is often inevitable in dealing with complex systems, all available variables were tested and these two were found to be significant.

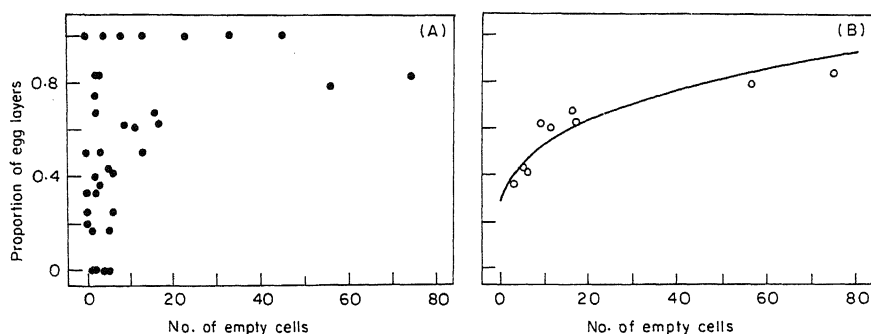


Figure 1. Scatter plot showing the proportion of egg-layers eclosing from each nest versus the number of empty cells on that nest (A). The two variables are significantly correlated as seen from Kendall's coefficient of rank correlation ($P < 0.005$; see table 4, row 5). The excessive scatter in the data is on account of our attempt to estimate the proportion of egg-layers for nests from which a very small number of animals were tested. The relationship between proportion of egg-layers and number of empty cells is seen more clearly when nests from which only 6 or fewer animals were tested are deleted (B). The solid line is computed using the equation $\ln Y = 0.2710 \ln(1 + X) - 1.2861$ which was fitted using the method of least square. $r^2 = 0.8501$. The slope 0.2710 has a standard error of 0.0430 and is thus significantly different from zero at $P < 0.0001$.

Complex systems often cannot be tackled by methods other than such 'fishing expeditions' but it may be argued that one's confidence in the role of any variable detected through such a method is not as high as it would be if its involvement were postulated beforehand on *a priori* grounds (see Martin and Bateson 1986, pp 134–135 for a discussion of this issue). For this reason an attempt was made to reconfirm these results both by performing additional statistical tests as well as by repeating the experiment and performing the original and new statistical tests on data from the new experiment and on data pooled from both experiments. In doing so, we were, as might be expected, confronted with minor discrepancies in the results between experiments and between statistical techniques. Here the rather conservative strategy of accepting only those results which were validated by all statistical techniques in at least one individual experiment and in the pooled data was adopted. In spite of such reconfirmation, our approach here was to first detect variables that may be important and then construct hypotheses as to their possible mode of involvement in pre-imaginal caste bias. It is for this reason that a conservative strategy which may lead to an error of rejecting an important variable rather than to the converse error of accepting an unimportant variable, was adopted.

Using this strategy, we reconfirm the association of the number of empty cells present on the parental nest and an animal's feeding rate during adult life with its probability of becoming an egg-layer. Such reconfirmation strengthens our model for pre-imaginal caste bias (Gadagkar *et al* 1988) which suggests that a declining influence of the queen leads to the accumulation of empty cells as well as to the production of daughters who are programmed to feed more and become egg-layers while a high influence of the queen leads to fewer or no empty cells and the production of daughters programmed to feed less and become workers. Thus caste is at least partly determined prior to eclosion even in a primitively eusocial wasp

such as *R. marginata* which lacks morphological differences between egg-layers and non-egg-layers.

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Dynamics of a population of *Ceriodaphnia cornuta* Sars (Crustacea: Cladocera) from a seasonal pond in Madurai

N MURUGAN

Department of Zoology, Madura College, Madurai 625 011, India

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Abstract. Investigation of the dynamics of a population of *Ceriodaphnia cornuta* indicated that the internal and external phenomena of clutch size and food supply were significant factors influencing population density and other population parameters. The relationships of clutch size with transparency and temperature were attempted. A single period of bisexual reproduction was noticed over a period of 12 months. The mean annual clutch size ranged from one to two eggs. Animals with 3 eggs were far fewer in percentage in the natural habitat. A low positive correlation of $r=0.27$ was recorded between mean brood size and body length, indicating that small body size naturally restricts the egg laying capacity of the females. The absence of size groups III and IV in the field samples was explained. The population parameters such as finite birth rate (B), instantaneous birth rate (b), population change (r) and death rate (d) were estimated from field data for this cladoceran.

Keywords. *Ceriodaphnia cornuta*; population density; composition; fecundity; size distribution; birth and death rates.

1. Introduction

This paper is one of a series describing behavioural aspects of zooplankton population in a eutrophic pond in Madurai. The population dynamics of *Ceriodaphnia cornuta* Sars are described and related to the environmental factors wherever possible. Cladocerans, particularly *Daphnia*, are ideal microcrustaceans for studying population characteristics in natural habitat since both eggs and adults can be collected quantitatively and relatively high biological responses in terms of fecundity to changing environmental conditions in a limited duration of time. There has been a paucity of information on the ecobiological studies of *C. cornuta* from Indian peninsular waters, the exception being the contribution of Michael (1962). Hence, an attempt has been made to investigate its population density and composition, fecundity and age structure in natural habitat.

2. Description of the pond

The investigation was carried out in a natural shallow pond located inside the campus of Madura College, Madurai (long. 78°8'E; lat. 9°56'N). The pond has an average depth of about 2 meters in the mid region. The depth decreases gradually towards the margin. The surface area covered by water is about 600 sq. meters. The trees belonging to the genera *Pongamia*, *Azadirachta* and *Morinda* give much stability to the margin of the pond and the decomposed leaves in the form of organic matter allow enormous growth of zooplankton. The absence of rooted vegetation is noteworthy. Microflora of the class *Cyanophyceae*, *Chlorophyceae*,

Bacillariophyceae and *Euglenophyceae* are recorded during the period of investigation. The free floating macrophytes of the family Lemnaceae appear during certain periods. *Microcystis*, *Coelospherium*, *Clastidium*, *Nostoc*, *Capsosira* among *Cyano-phyceae*; *Pandorina*, *Volvox*, *Polyedriopsis*, *Sphaerocystis*, *Ankistrodesmus*, *Eudorina*, *Scenedesmus*, *Desmids*, *Actinastrum* among *Chlorophyceae*; *Synedra*, *Cocconeis*, *Navicula*, *Tabellaria*, *Pinnularia*, *Cymbella* among *Bacillariophyceae*; *Euglena*, *Phacus* and *Trachelomonas* among *Euglenophyceae* are recorded genera of microflora.

3. Materials and methods

Samples were collected at weekly intervals between August 26, 1976 to July 15, 1977 from different regions of the pond. In total, 33 samples were collected covering a period of 12 months. To avoid errors caused by vertical migration of zooplankton if any, all samples were collected near mid day between 11:00 and 13:00 h. A 20 litre capacity collecting bucket was used for sampling. The samples were concentrated by filtration through a bolting silk sieve of 120 μ m mesh size. The zooplankton collected on a day was mixed and the concentrates were preserved in 4% formalin. In order to evaluate cladoceran population dynamics such as densities and composition, fecundity and size distribution were recorded as followed by Murugan (1989) for *Moina micrura* population. In addition, the length (the distance from the apex of the head to the base of spine) of parthenogenetic females with eggs was measured in each sample. The data obtained from the samples were analysed for birth rate, rate of population changes and death rate following Hall (1964) and George and Edwards (1974).

Physical and chemical parameters measured each week included pH, dissolved oxygen (Winkler technique), water transparency (Secchi disc) and temperature. Maximum and minimum temperatures were recorded by a thermister at 1 m deep.

4. Results

Seasonal variation in maximum and minimum temperatures of water and atmosphere and water transparency are represented in figure 1. The pH fluctuation was between 7.2 and 9.5. The day time oxygen concentration fluctuated around an average of 5 ppm.

Seasonal mean density values of *C. cornuta* with allied populations of Cladocera are shown in figure 2. Figure 3 illustrates seasonal variation in the mean length of mature females and mean egg number. Figure 4 shows the relationship between the body length versus egg number. Figure 5 is a graphical representation of different size classes of *C. cornuta*. Figure 6 represents instantaneous birth rate (*b*) and rate of population change (*r*) of *C. cornuta*.

Table 1 shows seasonal variation in the percentage composition of the population of *C. cornuta*. Field observation of the highest and the lowest egg numbers and lengths of adult females of tropical and temperate Cladocera are shown in table 2. Table 3 shows the laboratory observation of the highest and the lowest egg numbers and lengths of adult females of tropical Cladocera. Table 4 shows seasonal variation in the population data for *C. cornuta*.

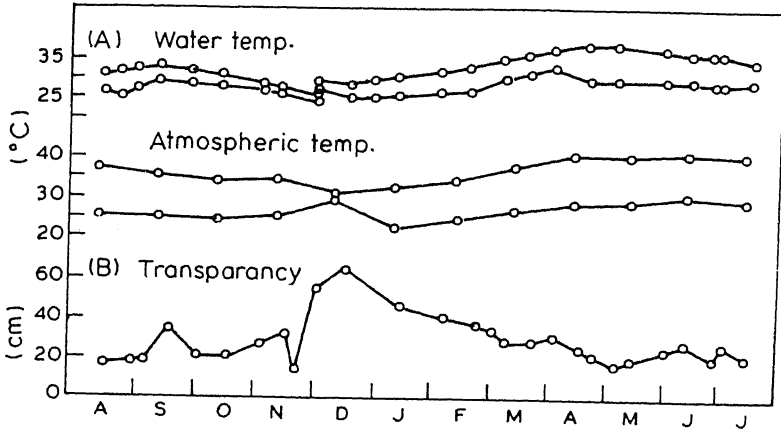


Figure 1. Seasonal variation in maximum and minimum temperatures of water and atmosphere and water transparency.

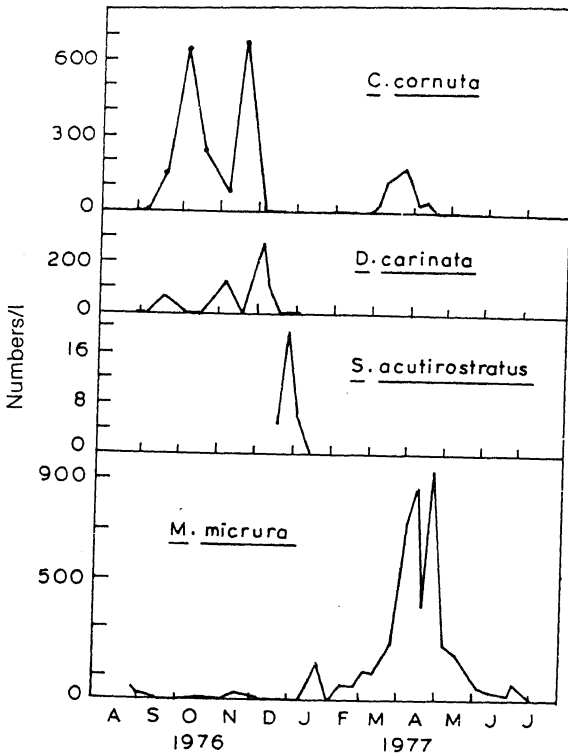


Figure 2. Seasonal mean density values of *C. cornuta* with allied Cladocera.

Discussion

1.1 Biology

The life span of *C. cornuta* is typical of many species of Cladocera. Under favourable environmental conditions reproduction occurs mostly by partheno-

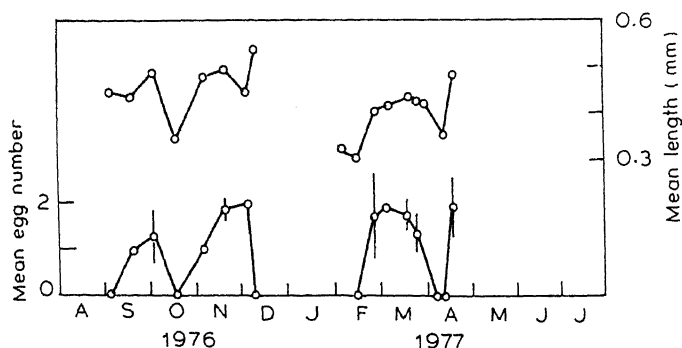


Figure 3. Seasonal variation in the mean length of mature females vs mean egg number.

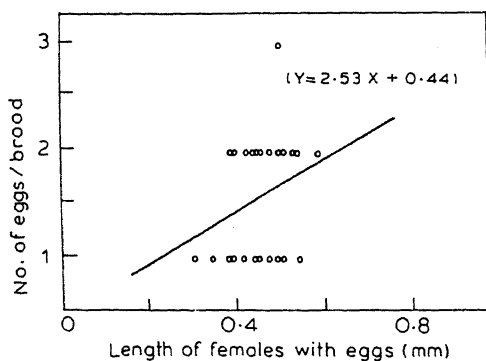


Figure 4. Correlation coefficient between body length and egg number.

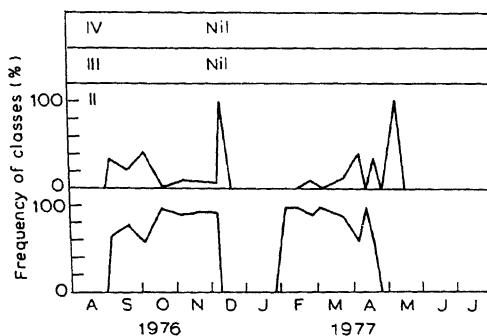


Figure 5. Seasonal variation of different size classes of *C. cornuta*.

genesis. Early experiments under laboratory conditions revealed that in a total life span of 21 days, this species produced 123.6 eggs in 18 clutches (Murugan 1975b). Michael (1962) observed that in a life span of 12 days, the same species released 42 eggs into the brood in 9 batches. The juveniles moult twice to reach the primiparous instar.

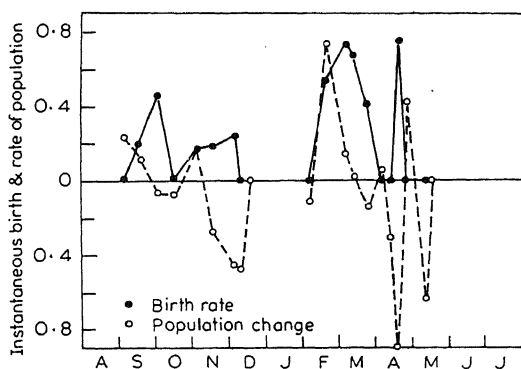


Figure 6. Instantaneous birth rate (b) and rate of population change (r) of *C. cornuta*.

Table 1. Seasonal variation in the percentage composition of the population of *C. cornuta*.

Date	Immature females (%)	Females of mature size without eggs (%)	Females with parthenogenetic eggs (%)	Ephippial females (%)	Males (%)	No. of individuals counted
1976						
26, 29 Aug.	0	0	0	0	0	0
5 Sept.	67	33	0	0	0	30
19 Sept.	78	20	2	0	0	50
3 Oct.	56	4	34	6	0	50
18 Oct.	98	2	0	0	0	50
6 Nov.	80	4	16	0	0	50
19 Nov.	88.75	0	8.75	2.5	0	80
5 Dec.	88.66	6.66	6.66	0	0	15
9 Dec.	0	100	0	0	0	1
18, 25 Dec.	0	0	0	0	0	0
1977						
3, 12, 17, 25 Jan.	0	0	0	0	0	0
4 Feb.	100	0	0	0	0	25
25 Feb.	60	40	0	0	0	10
3 Mar.	45	0	55	0	0	20
10 Mar.	48	1.3	50.6	0	0	75
23 Mar.	64	2	34	0	0	61
4 Apr.	60	40	0	0	0	5
11 Apr.	100	0	0	0	0	7
17 Apr.	44	4	52	0	0	25
24 Apr.	0	0	0	0	0	0
6 May	0	0	100	0	0	1
14 May	0	0	0	0	0	0
3, 12, 29 June	0	0	0	0	0	0
3, 15 July	0	0	0	0	0	0

5.2 Population density

The population of *C. cornuta* had two adjacent peaks in density: the first at the beginning of October (625 individuals/l) and the second at the end of November

Table 2. Field observations of the highest and the lowest clutch sizes of tropical and temperate Cladocera.

Species	Date and year	Mean maximum minimum clutch sizes	Mean length (mm)	Source
Tropical				
<i>S. acutirostratus</i>	26 Dec. 1976	28.9 ± 12.46	2.52	Murugan (1980)
	3 Jan. 1977	6.6 ± 2.07	2.01	
<i>D. carinata</i>	26 Aug. 1976	6.7 ± 2.25	2.15	Murugan (1980)
	6 Nov. 1976	1.0	1.72	
<i>C. cornuta</i>	5 Nov. 1976	2.0	0.44	Present study
	9 Sept. 1976	1.0	0.43	
<i>M. micrura</i>	12 Jan. 1977	4.4 ± 1.98	0.66	Murugan (1989)
	26 Aug. 1976	1.0	0.77	
Temperate				
<i>C. quadrangula</i>	1960	2.2	—	Smyly (1974)
	1965	3.0	—	
	1966	2.7	—	
	1967	2.0	—	
	1968	2.4	—	
	1969	2.3	—	

Table 3. Laboratory observations of the highest and the lowest clutch sizes of tropical Cladocera.

Species	Temperature (°C)	Mean maximum minimum clutch size	Mean length (mm)	Source
<i>S. acutirostratus</i>	28–30	27.0	3.1	Murugan and Sivaramakrishnan (1973)
		8.0	2.01	
<i>S. kingi</i>	28–30	20.5	0.79	Murugan and Sivaramakrishnan (1976)
		4.0	0.92	
<i>C. cornuta</i>	28–30	6.0	0.62	Michael (1962)
		3.0	0.43	
<i>C. cornuta</i>	28–30	9.3	0.70	Murugan (1975b)
		2.4	0.92	
<i>D. carinata</i>	28–30	16–18	2.0–2.2	Sumitra Vijayaraghavan (1970)
<i>M. micrura</i>	28–30	7.7	0.79	Murugan (1975a)
		3.0	1.00	

(650 individuals/l). The absence of this species from the middle of December to January seems to correlate with higher transparency, low temperature and scarcity of food. Low population amounting to about 175 individuals/l was recorded in March.

Two possible reasons for population peaks are attributed: the internal phenomenon of clutch size (figure 3) and the external phenomenon of food supply which was estimated by water transparency (figure 1B). The clutch size which is important in determining the total number of eggs in a population reached a

Table 4. Population data for *C. cornuta*.

	1	2	3	4	5	6	7	8
Date	N_o	N_A	\bar{E}	1/D	B	b	r	d
Sept. 5	300	99	0.00	1 day	0.00	0.00	0.23	-0.23
19	7250	1595	1.00	1 day	0.22	0.20	0.11	0.09
Oct. 3	31825	14003	1.34	1 day	0.58	0.46	-0.06	0.52
18	12150	243	0.00	1 day	0.02	0.02	-0.07	0.09
Nov. 6	3750	750	1.00	1 day	0.20	0.18	0.17	0.01
19	33225	3737	1.90	1 day	0.21	0.19	-0.27	0.46
Dec. 5	450	59	2.00	1 day	0.26	0.24	-0.45	0.69
9	75	75	0.00	1 day	0.00	0.00	-0.47	0.47
19	0	0	0.00	—	0.00	0.00	0.00	0.00
Feb. 4	280	0	0.00	1 day	0.00	0.00	-0.11	0.11
24	30	12	1.75	1 day	0.70	0.53	0.73	-0.20
Mar. 3	2350	1292	1.95	1 day	1.07	0.73	0.14	0.59
10	6075	3152	1.84	1 day	0.95	0.67	0.03	0.64
23	8475	3051	1.42	1 day	0.51	0.41	-0.14	0.55
April 4	1500	600	0.00	1 day	0.00	0.00	0.06	-0.06
11	2250	0	0.00	1 day	0.00	0.00	-0.30	0.30
17	500	280	1.96	1 day	1.09	0.74	-0.89	1.63
24	0	0	0.00	—	0.00	0.00	0.42	-0.42
May 6	150	150	0.00	1 day	0.00	0.00	-0.63	0.63
14	0	0	0.00	1 day	0.00	0.00	0.00	0.00

1. Total population size/50 litres.
2. Total adults (adults/50 litres).
3. Mean number of eggs/brood.
4. Rate of growth or development of eggs in a day which is reciprocal of duration of egg development.
5. Finite birth rate.
6. Instantaneous birth rate.
7. Observed instantaneous rate of population change.
8. Estimated instantaneous death rate.

maximum of two when the population was expanding and zero when the population was diminishing. In March in spite of its maximum clutch size the population of *C. cornuta* was not largely expanded. It may be explained by the fact that the population density of allied species of Cladocera (*M. micrura*) are high during this period.

The relationships of clutch size with water transparency and temperature were attempted to find out the influence of ecological factors on population. A comparison of figures 1B and 2 shows that the former relationship is understandable when the absence of eggs or depletion of *C. cornuta* at the time of higher transparency. Ingle *et al* (1937), Green (1956, 1966) and Hall (1964) agreed with the concept of clutch size and transparency relationship. de Bernardi (1974) and de Barnardi and Canali (1975) reported that populations of *Daphnia hyalina* show inverse relation with transparency both in Cannero and Ispra sampling stations of Lago Maggiore.

The relationship of clutch size to temperature by some authors (Tauson 1930; Green 1956; Hall 1964) was corroborated in this study. It is generally observed from figure 1A that fluctuations of maximum and minimum temperatures were between

24 and 35°C and 20 and 29°C respectively. Being a shallow body of water the temperature fluctuations are closely parallel to those of atmospheric temperatures. A comparison of figures 1A and 3 reveals the maximum clutch size at higher temperatures. It becomes obvious that temperature influences food production which in turn is responsible for larger broods. It has been reported that temperature effects on the development of population are less pronounced in deep lakes than in shallow lakes and ponds where hypolimnetic temperatures undergo considerable seasonal variations. Temperature increases with the progression of warm season are considerable and affect the entire mass, with much more pronounced and direct effect on growth rate. Therefore, temperature can affect a population in an indirect manner thus influencing the food availability which are always considerable (de Bernardi 1974).

5.3 Population composition

The percentage composition of population of *C. cornuta* was studied to determine the period of occurrence of bisexual reproduction and the extent to which females changed from parthenogenesis to producing resting eggs (Green 1966). Table 1 shows only one period of bisexual reproduction in the months of October and November. The percentage of ephippial females rose to 6 of the total population. The occurrence of ephippial females shows the instability of the population structure of *C. cornuta* in this ecosystem which is overall controlled by various causative factors such as food, temperature, crowding etc. This finding concurs with the observations of Murugan (1989) for *M. micrura* population. No males were recorded in the population of *C. cornuta*. Further, the absence of distinct sexual dimorphism in this species made the task of identifying males difficult. From this overall account it may be assumed that the low summer population of *C. cornuta* probably emerged from resting eggs in response to stimulus provided by the rise in temperature in the month of February. No females with resting eggs were found although each sample was searched carefully.

5.4 Seasonal variation in egg production and body length

The changes in the clutch size and the mean lengths of females followed the same pattern (figure 3) as in *M. micrura*. It has been reported that conditions which favour growth also favour egg production and the adult size and clutch size tend to fluctuate synchronously (Green 1966; Murugan 1989).

The mature females of this species formed a low percentage of 27.74 of the total population. The mean annual clutch size ranged from 1 to 2 eggs. The animals with clutch sizes of two eggs were found more frequently than the other and they formed 65% of the total population. Those with clutch sizes below 2 formed 33%. The animals with 3 eggs were far fewer in number than the others and amounted to about 1.78%.

The mean annual clutch size of related genus of a temperate form *C. quadrangula* (Smyly 1974) varied from 2 to 3 eggs which in this respect is more or less similar to that of *C. cornuta* (table 2). It has been reported that the most frequent occurrence of the clutch size in *C. quadrangula* was two eggs. A grand mean egg number of the similar genera (*C. pulchella*, *C. reticulata*, *C. megalops* and *C. laticaudata*) from

temperate region was 3.83, 5.20, 3.85 and 2.76 respectively (Burgis 1967). Table 3 depicts the highest and the lowest clutch sizes of tropical Cladocera reared under laboratory conditions. The highest clutch was noticed in *Simocephalus acutirostratus* (Murugan and Sivaramakrishnan 1973) and the lowest in *C. cornuta* (Michael 1962). The largest individual clutch size of *C. cornuta* was 9.3 eggs (Murugan 1975b).

5.5 Mean brood size vs body length

In the natural habitat the length of the females carrying parthenogenetic eggs were varying from 0.3–0.6 mm (figure 4) in *C. cornuta*. The correlation coefficient worked out for the above relationship showed a low positive correlation of $r=0.27$ and the regression equation is $Y=2.53 X+0.44$. It becomes evident that the egg number increases with the body length less rapidly since the body size of the female carrying eggs are in a narrow range. The statement that small body size naturally restricts egg laying capacity of the female concurs with the earlier observation in *M. micrura* by Murugan (1989). Several workers (Anderson *et al* 1937; Anderson and Jenkins 1942; Green 1954, 1956; Richman 1958; Burgis 1967; Buikema 1973) have observed correlation between the size of the organisms and the number of eggs produced by them. Lei and Clifford (1974) also found a significant positive correlation in the laboratory cultures of *D. schodleri*.

5.6 Size distribution

The knowledge of the distribution size of *C. cornuta* is essential for analysing the changes in the population. Different classes were recognised after studying the ontogenic development of *C. cornuta* under laboratory conditions (Murugan 1975b). The following size groups have been used.

Class	I	<0.47 mm.
Class	II	0.47–0.70 mm.
Class	III	0.70–0.75 mm.
Class	IV	>0.75 mm.

The lower limit of class II was found to correspond to the carapace length of the primiparous instar. The lower limit of the class III corresponds to the carapace length at which maximum number of eggs were noticed in the brood under laboratory observations. Changes in the frequency of each class are shown in figure 5.

First two size groups are only represented in the population as evidenced in which length of females carrying eggs ranged between 0.3 and 0.6 mm. It may be assumed that the first generation which appeared in September took about nearly 4 months to reach maturity (class II). The peaks of population coincide with maximum proportion of premature individuals rather than mature forms as in *M. micrura* (Murugan 1989). This observation concurs with the findings of George and Edwards (1974) in *D. hyalina*. The development of second cycle can also be traced by successive two-size classes. However, the other two classes (III and IV) were absent in the field throughout the period of study and were recognised only by rearing in the laboratory. A logical explanation that could be made is that the growth of this species is somehow impaired probably due to the presence of

D. carinata which is a more efficient filter feeder in the same niche. Parker (1958) stated with reference to fish population that number of individuals may continue to increase in the presence of limited food supply but each individual becomes stunted in size. This has indeed been true with *Daphnia*.

5.7 Birth rate, rate of population change and death rate

The observed instantaneous rates of population change (r) calculated from successive pairs of population density values, the instantaneous birth rates (b) calculated from the time of development of eggs inside the brood, are given in table 4. The estimated instantaneous death rates (d) which are obtained by subtracting the interpolated values of r from b for each sample, include natural mortality.

The maximum positive r value was 0.73 and the minimum value of 0.03 was recorded during the period of investigation. The mean positive b value was 0.02 and the maximum value of 0.74 was recorded at the middle of April. A maximum value of 1.63 was estimated as instantaneous death rate. Recalling the density changes (figure 2) it becomes evident that the period of higher birth rates in the months of September and November correspond to the maximum density of *C. cornuta*. The birth rates are entirely realised for population increase. However in February and March large increase in birth rate did not cause an increase in population. Instead, ' r ' diminished very rapidly and death rate (d) also increased. This unrealistic potential population can be understood by comparing the values of b and r in figure 6. It is generally observed that *C. cornuta* has higher birth rate during high temperature in which respect this resembles *D. schodleri* (Wright 1965). The average values of birth rate, rate of population change and death rate for *C. cornuta* were 0.22, 0.092 and 0.34 during 1976–1977. It would be still premature to give a definite interpretation of these results which should be the main object of more detailed experimental research in this tropical peninsular India.

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Biofilm characteristics in coastal waters of Bombay

R B SRIVASTAVA, S N GAONKAR and A A KARANDE

Biology Division, Naval Chemical and Metallurgical Laboratory, Tiger Gate, Bombay 400 023, India

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Abstract. Field and laboratory studies were carried out to analyse the development of biofilms generated on various metallic and non-metallic coupons immersed in coastal waters of Bombay. It is observed that the nature of the biofilm formed not only varies from substratum to substratum but is also influenced by the quality of water, flow conditions and the biotic status of the seawater. The biofilms depending upon the degree of entrapment of the inorganic detritus have varying weights. The biofilms formed on metallic surfaces when compared with perspex surface, are both qualitatively and quantitatively richer. Cupro-nickel, despite its toxicity, supports denser film than the one developed on inert perspex surface. The metallic coupons, both inert and toxic, support thicker films in polluted waters than in clean waters.

Keywords. Biofilm; biodeterioration; marine metals.

1. Introduction

Materials exposed to natural seawater are readily covered by both organic and inorganic detritus, bacteria, diatoms and their extracellular breakdown products. In the recent past several cases of marine engineering failures attributed to the deposition of biofilms have been reported (Purkiss 1972; Hanger 1975; Connan 1984; Hamilton 1985; Maxwell *et al* 1987; Morgon and Steele 1987). Biocorrosion induced by sulfate reducing bacteria (SRB) residing under biofilm has particularly been recognised as a serious maintenance problem.

Depending upon environmental conditions and the available nutrients, biofilm may contain a wide range of aerobic, facultative and anaerobic microorganisms that together hydrolyse and ferment carbohydrates, proteins and other primary nutrients (Hamilton and Maxwell 1986). The information on biofilm development in tropical waters is lacking and the behaviour of metals like titanium, stainless steel, aluminium-brass and copper-nickel alloys, all of marine engineering interest, is unknown. This paper analyses the nature of biofilms developed on marine materials immersed in coastal waters of Bombay (lat. 18° 55' N, 72° 50' E).

2. Materials and methods

Two sites in Bombay harbour were selected for studying the nature of the biofilm generated on metallic test coupons and pipes. The first site was an open water having good tidal flushing, low (10–15 ppm) biochemical oxygen demand (BOD) and high (4.5 ppm) dissolved oxygen. The second site was a semienclosed water close to the shore receiving untreated organic sewage and therefore having a noticeable growth of SRB (Srivastava and Karande 1986).

The metallic coupons of titanium, stainless steel, cupro-nickels (70:30 and 90:10) and aluminium-brass as well as of perspex of the size 15 × 10 cm were immersed one

metre below the water surface. The metallic coupons for the exposure were prepared by adopting the method reported by Gerchakov *et al* (1976). The coupons were polished by finer grades (220, 320, 400 and 600) of emery paper, washed in detergent, rinsed with distilled water, dehydrated in methanol, weighed and stored in a dessicator until immersed at the sites.

For studying the biofilm generated on metallic coupons and tubular specimens under 'once through' flow conditions, a seawater exposure assembly was employed (Karande 1987). The assembly was fitted on a pontoon moored in harbour waters for carrying out field experiments.

The films were examined for their physical, chemical and biological characteristics. Wet film thickness of the biofilm was measured by using light section microscope (Little 1980). For film developed in tubular specimens, the thickness was measured by calculating the volume of water displaced by the film (Characklis *et al* 1982). The biofilm from an area of 25 cm² was removed from each metallic surfaces with the help of a non-metallic (teflon) spatula. For the estimations of the total dry weight of the film as well as for estimations of organic and inorganic contents, the methods adopted by Mollica *et al* (1984) were followed. A method recommended by Dubois *et al* (1956) was employed to calculate the total carbohydrates in the film. The lipid phosphate content of the biofilm was estimated by following the method of White *et al* (1979). The films were however, macerated to extract these biochemicals for colorimetric estimations. Modified Zobell marine agar, Difco 2216 (Gerchakov *et al* 1976) was used for counting and isolating bacteria employing pour plate method. The total and acid producing bacterial colonies growing on this medium were counted after one day, 4 days and again 14 days of incubation at 37°C. Diatoms present in the film were enumerated by adopting the procedure of Patrick and Reimer (1967). The extent of metal loss due to corrosion in the presence of biofilm was assessed by estimating the weight loss. The tubes and coupons before weighing were treated with cleaning solution for the removal of corrosion products. Titanium was cleaned with 1:1 warm sulphuric acid whereas cupro-nickel tubes and coupons were treated with a mixture of sulphuric acid and hydrochloric acid (1:5) as per the American Standard Test Methods (ASTM). Specimens were rinsed following cleaning and rubbed lightly with a bristle brush under running water. Thereafter tubes and coupons were dried and weighed.

3. Results

3.1 Biofilm on perspex

Figure 1 illustrates the biofilm build up in terms of wet film thickness at varying time intervals on exposed perspex coupons. It is seen that within the first 24 h, thicknesses of the films formed are variable and equilibrium is attained thereafter. At the end of 10 days period, a film thickness in the range of 60–100 µm is observed.

Table 1 summarises the biofilm characteristics on perspex coupons exposed in clean and polluted waters. Table 2 gives characteristics of these sites. It is noted that the films in terms of their thicknesses do not show any notable differences in clean and polluted waters on perspex material. It is also observed that growth of the films on perspex coupons does not exceed beyond a certain thickness irrespective of the quality of water wherein they are immersed. Contrary to expectation, weight of

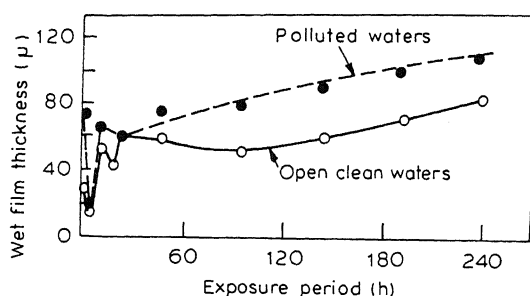


Figure 1. Biofilm build up rate (thickness in μ m) on perspex coupons at varying time intervals.

Table 1. Biofilm characteristics on perspex coupons immersed in harbour waters for 15 days.

Biofilm characteristics	Study stations	
	Open clean waters (range)	Polluted waters (range)
Wet film thickness (μ m)	57-111	74-122
Total dry weight (mg/cm^2)	0.13-1.16	0.45-2.40
Organic content (%)	26-32	25-40
Diatoms (average no./ cm^2)	7.2	27.2
Bacteria (no. $\times 10^3/\text{cm}^2$)	4-69	7-160
Total carbohydrates ($\mu\text{g}/\text{cm}^2$)	0.57-2.50	0.66-2.80
Lipid phosphate (nmol/cm^2)	8.8-19.4	9.9-19.8

Table 2. Seawater characteristics at two harbour sites in Bombay.

Sea water quality	Sites	
	Open waters	Tidal basin waters
Temperature ($^{\circ}\text{C}$)	24.8-30.3	24.6-30
pH	7.2-8.03	7.1-7.7
Salinity (%)	36.2-36.8	33.2-37.7
Dissolved oxygen (mg/l)	3.37-6.08	0.74-2.74
Biochemical oxygen demand (mg/l)	12.25-19.4	37.9-56.8
Hydrogen sulphide (mg/l)	24.9-42.6	52.1-102.5
Total number of bacteria ($\times 10^3/\text{ml}$)	4-12	18-41
Total suspended particulate matter (mg/l)	14.0-46.33	5.0-37.6

the inorganic fraction of the film formed in the waters receiving inadequately treated sewage was found to be higher than the organic weight. The diatom population being rich in these waters, their frustulae seem to contribute to inorganic weight of the film. The bacterial population as expected was found to be rich but bacteria did not seem to contribute notably to the organic weight of the film formed.

3.2 Biofilm on metallic surfaces

Table 3 summarises the data on biofilm build up on titanium, stainless steel and perspex exposed for varying periods. It is observed that at the end of 40 days

Table 3. Build up of biofilm on materials immersed in harbour waters from 3, 10, 30 and 40 days.

Materials and period of exposure	Biofilm characteristics			
	Total number of bacteria ($\times 10^4/\text{cm}^2$)	Total dry weight (mg/cm^2)	Combustible organic matter (mg/cm^2)	Total nitrogen ($\%/100 \text{ mg}$)
3 days				
Titanium	0.03 (0.003)	0.06 (0.002)	0.03 (0.003)	0.66 (0.03)
Stainless steel	0.02 (0.005)	0.05 (0.003)	0.03 (0.001)	0.83 (0.02)
Perspex	0.08 (0.013)	0.09 (0.01)	0.03 (0.002)	2.5 (0.2)
10 days				
Titanium	0.24 (0.05)	0.17 (0.013)	0.1 (0.03)	1.56 (0.06)
Stainless steel	0.13 (0.01)	0.22 (0.012)	0.07 (0.015)	1.0 (0.3)
Perspex	0.98 (0.13)	0.14 (0.013)	0.07 (0.011)	2.0 (0.15)
30 days				
Titanium	68.0 (2.7)	4.03 (0.11)	0.92 (0.2)	1.5 (0.07)
Stainless steel	36.0 (2.3)	4.63 (0.51)	0.67 (0.06)	1.5 (0.04)
Perspex	7.2 (1.4)	0.86 (0.07)	0.27 (0.02)	1.25 (0.03)
40 days				
Titanium	88.0 (3.5)	16.0 (1.5)	1.81 (0.09)	1.5 (0.13)
Stainless steel	75.0 (2.3)	16.7 (0.5)	1.65 (0.04)	1.5 (0.15)
Perspex	25.0 (3.4)	2.5 (0.4)	0.55 (0.09)	2.5 (0.18)

Numbers in parentheses show SD ($n = 12$).

exposure, a highest number of bacteria is noted on titanium followed by stainless steel. The total dry weight of the film and its organic component increase with increasing exposure periods. The biofilm developed on metallic material eventually grows richer than that on non-metallic material like perspex.

Table 4 gives biofilm characteristics in respect of bacteria on titanium, stainless steel, aluminium-brass, copper-nickel (70:30) and copper-nickel (90:10). It is observed that irrespective of the sites, the numbers of bacteria on inert metals are more than those on cupro-nickel coupons. Figure 2 illustrates the variations in bacterial and diatom populations on different metallic surfaces in clean and polluted harbour waters. Figure 3 illustrates copper-nickel (70:30) surface being fouled with rod shaped bacteria.

Table 5 reveals that both titanium and stainless steel in clean waters have generally thicker wet films than on copper base alloys. In polluted waters also the growth of biofilm is richer in terms of its thickness. It is further observed from table 5 that the inorganic content of the film on any surface and in any of the two environments is always more as compared to organic content. However, the inorganic content is strikingly more on cupro-nickels and this could be attributed to their surface corrosion products.

3.3 Biofilm generation under flow conditions

Table 6 shows biofilm growth on internal surfaces of copper-nickel (70:30) straight and 'U' tubes exposed to running seawater in the laboratory. It is observed that straight tubes generate more biofilms than the 'U' tubes.

Table 4. Bacterial populations in biofilm on metallic coupons immersed in harbour waters for 7 days.

	No. of Bacteria	No. of acid producers	No. of bacteria	No. of acid producers
	($\times 10^3/\text{cm}^2$)			
Marine metal and alloys	Open clean waters		Polluted waters	
Titanium	36 (3)	6.7 (0.9)	72 (3.8)	8.2 (0.7)
Stainless steel	32 (2)	3.5 (1.2)	61 (4)	5.3 (0.4)
Aluminium-brass	21.3 (4.9)	3.8 (1.3)	55.1 (6.2)	6.2 (1.6)
Copper-nickel (70:30)	20 (3)	3.5 (0.4)	48 (3.7)	3.7 (0.9)
Copper-nickel (90:10)	13.3 (1.8)	1.2 (0.4)	40.8 (2.6)	1.8 (0.5)

Numbers in parentheses show SD ($n = 12$).

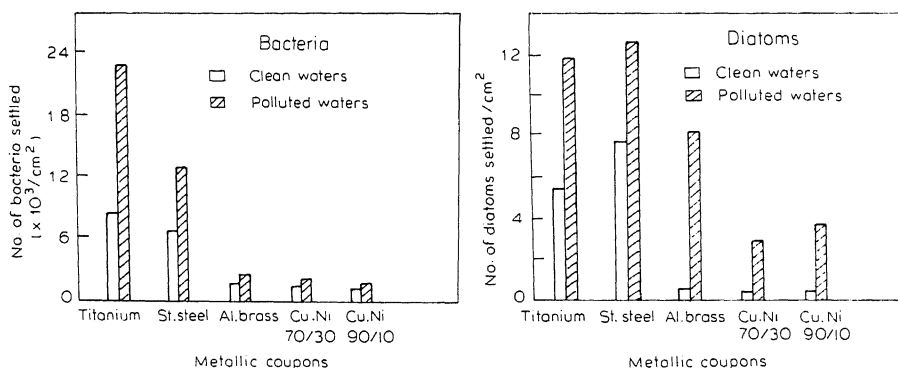
**Figure 2.** Bacterial and diatom populations on metallic coupons exposed in harbour waters for 15 days.

Table 7 summarises the data on film formation on titanium and copper alloy sheet coupons as well as in tubes exposed to running seawater at the two harbour sites for a period of 130 h. Both coupons and tubes show thicker films in polluted waters as compared to cleaner waters. It is, however, noted with interest that unlike quiescent waters (tables 4 and 5), under flow conditions aluminium-brass and cupro-nickel develop thicker films than those on titanium surfaces. Under flow conditions the films formed on sheet coupons of any metals are richer than those formed on tubular surfaces. This is evident by higher bacterial counts as well as by higher values of both total carbohydrates and lipid phosphate noted in films harvested on sheet coupons. In quiescent waters, the films formed on inert surfaces are thicker and stable, whereas under flow conditions they are reduced because of their sloughing-off from the substrates. On copper surfaces, on the other hand, the films formed are not easily dislodged.

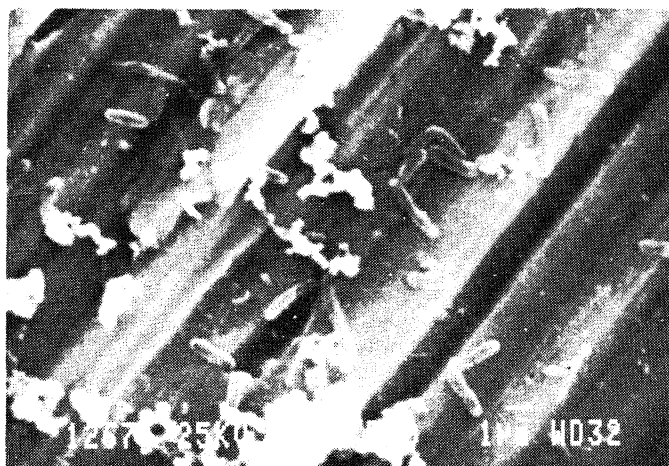


Figure 3. SEM photograph of copper-nickel 70:30 coupon exposed for a period of 4 days. Note bacteria.

Table 5. Biofilm characteristics on metallic coupons immersed in harbour waters for 15 days.

	Marine metals and alloys				
	Titanium	Stainless steel	Aluminium-brass	Copper-nickel (70:30)	Copper-nickel (90:10)
Thickness (μm)					
Open clean waters	160 (18.88)	150 (19.61)	120 (13.31)	100 (20.67)	140 (25.56)
Polluted waters	240 (14.81)	200 (22.07)	145 (21)	160 (22.84)	165 (12.43)
Dry weight (mg/cm^2)					
Open clean waters	2.7 (0.64)	2.52 (0.59)	1.71 (0.17)	1.4 (0.67)	2.2 (0.56)
Polluted waters	2.8 (0.35)	2.7 (0.52)	2.9 (0.95)	2.41 (0.14)	3.02 (0.81)
Ratio of organic/inorganic contents in biofilms					
Open clean waters	0.92	0.53	0.42	0.31	0.33
Polluted waters	0.42	0.34	0.33	0.17	0.13

Numbers in parentheses show SD ($n = 12$).

Table 6. Biofilm growth in tubular copper-nickel (70:30) under 'once through' laboratory conditions*.

Biofilm characteristics	Metal configuration	
	Straight tube	Bent (U) tube
Wet film thickness (μm)	40.7 ± 0.28	16.8 ± 0.4
Dry weight (mg/cm^2)	3.42 ± 0.25	2.34 ± 0.19
Total carbohydrates ($\mu\text{g}/\text{cm}^2$)	2.03 ± 0.47	0.77 ± 0.37
Lipid phosphate (nmol/cm^2)	5.3 ± 0.4	2.6 ± 0.3

*Water velocity 3 ft/s, continuous flow for 48 h.

The biofilms generated on copper base alloys unlike those on titanium and stainless steel were hard, tenacious and difficult to remove, particularly so in

Table 7. Characteristics of biofilms formed on metallic coupons and tubes exposed to 'once through' flowing seawater for 130 h (velocity 5 ft/s).

	Material					
	Titanium		Copper-nickel (70:30)		Aluminium-brass	
	Coupons	Tubes	Coupons	Tubes	Coupons	Tubes
Wet film thickness (μm)						
Open clean waters	27.55 (4.41)	22 (0.7)	39.06 (2.56)	33.2 (2.4)	50.22 (2.82)	42.2 (1.7)
Polluted waters	41.29 (1.09)	35.5 (2.7)	68.11 (1.41)	62.3 (1.3)	101.52 (6.68)	78.2 (5.3)
No. of bacteria ($\times 10^3/\text{cm}^2$)						
Open clean waters	22.2 (4.45)	3.71 (1.24)	17.11 (2.81)	2.45 (0.01)	18.33 (2.83)	2.55 (0.24)
Polluted waters	43.1 (3.4)	18.2 (2.9)	86.8 (7.3)	7.95 (3.17)	32.3 (3.6)	8.85 (2.62)
Total carbo/hydrates ($\mu\text{g}/\text{cm}^2$)						
Open clean waters	15.94 (2.74)	3.75 (0.57)	7.97 (0.25)	2.41 (0.6)	13.37 (0.95)	0.89 (0.11)
Polluted waters	17.97 (2.77)	5.31 (1.57)	14.17 (1.97)	3.2 (0.28)	17.44 (0.76)	3.53 (0.63)
Lipid phosphate (nmol/cm^2)						
Open clean waters	152.71 (16.5)	77.42 (9.78)	112.25 (3.95)	35.39 (6.82)	142.86 (14.56)	40.93 (5.4)
Polluted waters	263 (9)	136 (18)	211 (13.7)	109 (11)	248 (4)	121 (12.2)

Numbers in parentheses show SD ($n = 12$).

polluted waters. Aluminium-brass surfaces in polluted waters became tanned black and lacked patina colour observed in cleaner waters. Cupro-nickel surfaces also developed black coatings in polluted waters unlike brown colour observed in cleaner waters (figure 4).

Table 8 gives loss in weight of tubes due to corrosion for 3 metals exposed at two sites in a flowing seawater. It is observed that the losses in weights of cupro-nickel (70:30) and aluminium-brass due to corrosion are 3–5 times more in polluted waters than in cleaner waters.

4. Discussion

The analysis of the biofilms generated on non-metallic surface like perspex, primarily carried out to identify the gross composition of the film, reveals that it is mostly comprised of inorganic detritus. This is observed irrespective of the nature of water quality in which the microfilms are harvested. That inorganic matter is usually more than the organic fraction has been noted in several earlier reports (Berger and Little 1980). Even in polluted water containing organic effluents, the inorganic component in the film is more. In polluted waters such as the present one, increased proportion of inorganic matter is due to rich presence of periphytic

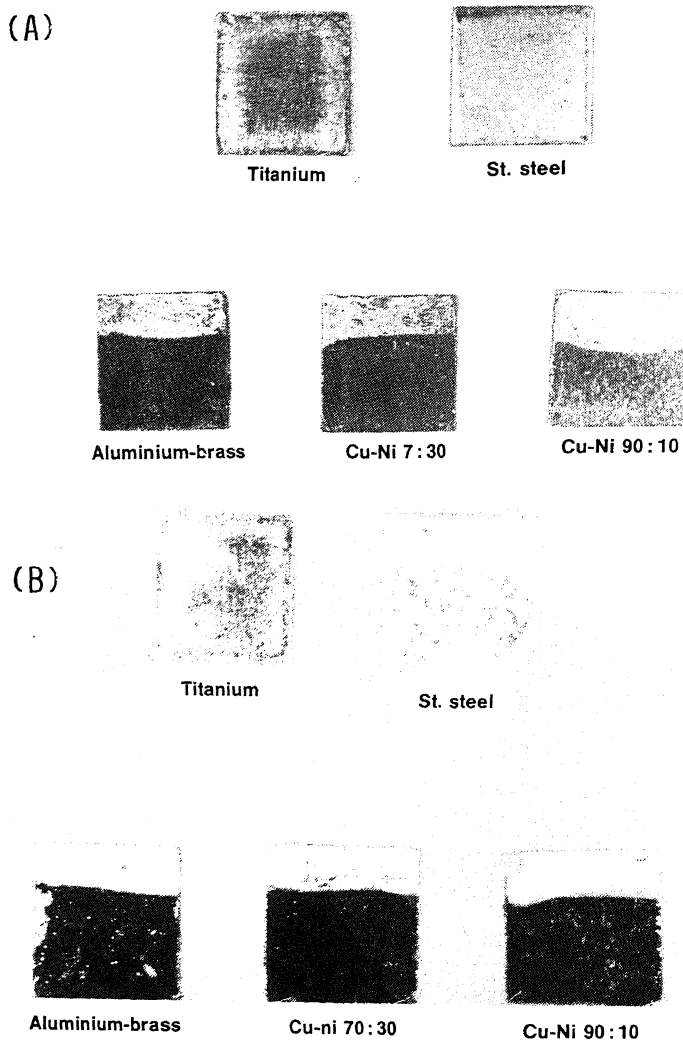


Figure 4. Test coupons exposed in (A) open clean and (B) polluted waters for a period of 15 days.

diatoms. Bacteria on the other hand, even if present in large numbers, do not contribute to organic content of the film. This is also reported earlier by Berger and Little (1980) in their ocean thermal energy conversion related biofilm studies.

The biofilms depending upon the degree of entrapment of the inorganic detritus have varying weights. There exists no correlation between the thickness and weight of the biofilm. The growth of the film on perspex, in terms of its thickness does not progress beyond a certain limit.

When compared with perspex surface, the biofilms formed on metallic surfaces are both qualitatively and quantitatively richer. This is particularly noticeable with regard to inert metals like titanium and stainless steel. The cupro-nickel surfaces despite their toxicity, also gather more film than the inert perspex surface.

Table 8. Weight loss of fouled metallic tubes carrying flowing seawater*.

Marine metals and alloys	Biofilm weight (mg/cm ²)		Weight loss of metals (mg/cm ² /day)	
	Open clean waters	Polluted waters	Open clean waters	Polluted waters
Titanium	0.55 ± 0.35	1.1 ± 0.03	ND	ND
Copper-nickel (70:30)	1.29 ± 0.16	3.96 ± 2.66	0.147 ± 0.023	0.396 ± 0.134
Aluminium-brass	1.26 ± 0.21	5.43 ± 1.2	0.125 ± 0.043	0.656 ± 0.154

*Water velocity 5 ft/s, continuous flow for 130 h.

ND, Not detectable.

Gerchakov *et al* (1978) observed that bacteria do attach to copper surfaces and produce copious quantities of polymer. Little (1980) got evidence to suggest that the cupro-nickel surfaces were not immune to bacterial attack. A lack of good growth on perspex can be explained by the fact that 'adhesion of organisms to a surface might be influenced by the hydrophilic or hydrophobic characteristics of the surface, the surface charge and the chemical functional groups available for reaction' (Kristoffersen *et al* 1982).

The impact of polluted water on the growth of the film quantified in terms of its thickness is not readily apparent on perspex coupons. However, the metallic coupons, both inert and toxic, support thicker films in polluted water than in clean waters. In case of inert metals, this increase is not found to be due to corrosion products and therefore can be attributed to the suspended sediments and to the frustulae of the diatoms which occur in large numbers at the study station. Besides the diatoms, it is possible that bacteria also indirectly contribute to the increase in inorganic fraction of the film by providing glycocalyx matrix for the entrapment of the suspended matter. White and Benson (1984) observe that extracellular biopolymers give sticky consistency due to the uronic acids. Ford *et al* (1987) have also suggested that the microbial exopolymers have ability to bind metal ions.

The bacterial populations in the films on titanium and stainless steel coupons were richer than in cupro-nickel films. The numbers of bacteria including acid producers observed to be more on coupons exposed in polluted waters than those in cleaner waters. In our study, an enhanced corrosion of cupro-nickel as judged by the weight loss was observed in polluted water. Gerchakov *et al* (1976) have suggested that the acid producing bacteria contribute to corrosion of metals.

The present study shows that the development of the biofilm is also influenced by the shape and geometry of the material. For instance in running seawater generally the early film formation was richer on sheet coupon surface than in tubular pieces and that the growth was better in straight tubes than that noted in the bent tubes. It is further observed that inert surfaces harvest thicker films in quiescent waters but in flowing seawater the films formed on cupro-nickel surfaces are thicker and stable. Characklis (1982) who examined the progress of biofouling deposition on copper nickel and titanium in a laboratory seawater system, observed that 'if only total deposit mass is considered, the copper-nickel fouled to a much greater extent than titanium'. He further noted that 'consideration of volatile deposit mass suggests that biofouling was essentially the same in the two alloys'. In our studies also we

observed that the biofilm deposition particularly on aluminium-brass to be more than on titanium under flow conditions and the combustible organic matter as depicted by total carbohydrates and lipid phosphate values was the same for the two surfaces. An increased biofilm growth on aluminium-brass observed by us therefore is due to surface corrosion products. Both copper-nickel and aluminium-brass thus show increased biofilm weights as compared to titanium.

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Foreword

Insects and plants interact with each other in a myriad ways. Many interactions involve mutual adaptations that enable the survival of the participants. Selection pressures are provided by plant defenses for counteradaptation by insects, which in turn select for modified defense in plants. Allelochemicals, which are secondary plant products, are known to have been evolved by plants as a response to attack by insects, and as the plants evolved, some of the associated insects also evolved with them. Exploitation of closely related plant families was made possible through changes in digestive processes, enzymes and other factors, leading to the evolution of specific differences among insects and resulting eventually in increased specialization within a group. We now have a better understanding of the role of sensory physiology of insects as one of the factors that determine susceptibility or resistance of plants to attack by insects. The association between insects and their host plants tends to be dynamic, involving mutual adaptations resulting in phytochemical diversity on the one hand and tolerance of insects to the chemical defenses of plants on the other. The host plant determines the survival, growth and multiplication rates of the concerned insects. The most important barrier to overcome is the behavioural one; the chemosensory mechanisms of insects respond differently to different chemicals, enabling the insects to locate and identify host plants, so that food discrimination is based on visual and olfactory stimuli. Thus insects encounter their host plants after processing the sensory information received from them and making appropriate movements that increase the probability of establishing and maintaining contact with the right food source. Each of the major steps in host plant selection is influenced by the chemical composition and the nutritional quality of the host plant as well as the nature of the secondary metabolites that play a major role in the attraction or repulsion of the insects. In order to successfully utilize its host plant, the insect must be versatile enough to complete the normal sequence of events; failure at any one step may confer an advantage to the host plant. The papers presented in this issue of the *Proceedings (Animal Sciences)* are based on a series of specialist lectures delivered at the DST National Workshop for young scientists held at the Entomology Research Institute from 22 July 1989 to 7 February 1990. The papers relate to resistance mechanisms of plants to insects, role of insects in wheat control, impact of natural products, and bioenergetics and biosystematics in relation to insect-plant interactions, to mention some of the more important topics.

The Workshop took into consideration all aspects relating to insect-plant interactions. This is a growing interdisciplinary field involving entomologists, botanists, biochemists and the plant breeders. Biological control also needs to be understood on the basis of the tritrophic relationship involving insect, host plant and parasite. The present decade as well as the next century will witness the increasing relevance of biotechnology in the study of insect-plant interactions, since future avenues of research will involve the identification and characterization of resistance genes, the development of probes to detect resistance genes and the transfer of resistance genes both within and between species.

Facets of chemical ecology in insect-plant interactions: An overview

T N ANANTHAKRISHNAN

Entomology Research Institute, Loyola College, Madras 600 034, India

Abstract. An overall analysis of the complex interrelationships involved in insect-plant interaction has been attempted with particular reference to the mechanisms of host plant searching and acceptance, nutritional and non-nutritional factors, defense systems in plants and insects and recognition systems in plants to invasion by foreign organisms.

Keywords. Chemical ecology; insect-plant interaction; host selection; plant defence capability; wounding in plants; chemical defenses.

1. Introduction

The ability of phytophagous insects to utilize plant tissues for energy, growth and reproduction has made them targets for the serious economic losses sustained, and the resultant reduction in agricultural productivity. The diversity of their feeding habits on leaves, buds, stems, roots and seeds, combined with their ability to act as vectors of bacterial, fungal and viral diseases have been basically responsible for the losses sustained. The basic question involved is: Are plants passive to such depredations? if not, how do they react to insect attacks? What are the mechanisms of defense and if the plants tend to be resistant or tolerant, how do insects overcome the defenses? These are some of the fundamental questions that have cropped up over the years and the subject of 'chemical ecology' has come to the forefront in providing a convincing answer to problems concerning insect-plant interactions, an understanding of which is undoubtedly the basis for solving problems of applied ecology. In an attempt to analyse the complex relationships involved, this overview envisages to identify in a very brief and general way such aspects as mechanisms of host plant searching and acceptance, nutritional and non-nutritional factors, defense systems in plants and insects and finally recognition systems in plants to invasion by foreign organisms. Incidentally mention may be made of the involvement of coevolutionary factors in these interactions, so that as the plants tend to diversify and develop morphological and physiological defense systems, insects also tend to develop detoxifying mechanisms (Labeyrie *et al* 1987). Co-evolutionary mechanisms are very evident in gall-forming insects where the tendency towards specialization increases with increased intimacy between the insect and host plant (Ananthakrishnan 1983, 1984). The gall formers are generally specific to a single host and they modify plant growth, live within plant tissues for most part of their lives. Gall formation has been known to be a genetic commitment towards adaptation of exploitation of plant species. It is only through the unravelling of the diverse defense mechanisms that suitable techniques could be developed to control insects. Both plants and insects being dynamic systems, the complexity of age-correlated biochemical processes has made the study of insect-plant interactions a really challenging subject.

2. Basis of host selection

Host plant selection behaviour involving habitat selection and discrimination

mechanisms between host species is known to play a significant role in insect-plant interactions. With plant species and individuals tending to vary in quality or suitability for insect growth, reproduction and survival, insects in turn tend to avoid plants of low quality, preferring to feed on those of high quality. This has led to aspects pertaining to the behavioural responses of insects to their food plant quality variation. Behavioural mechanisms tend to restrict their searching to habitats containing host plants. Insects are endowed with an array of sensory capabilities, chemosensory, mechanosensory, visual etc. By visual and chemical cues they seek a suitable habitat and are attracted to the host plant by chemical stimuli, and the olfactory and gustatory sensilla enable detection of these stimuli resulting in the acceptance or rejection of the host plant. The sensilla concerned with chemoreception are variously distributed, but are more abundant on the antenna mouthparts and legs and involve different types such as sensilla trichodea, chaetica, campaniformia etc., whose receptor cells enable transfer of information for the external environment to the central nervous system, for necessary behavioural action. Recognition and preference of host plants involve the integration of a complex of neural and metabolic events such as nutritional factors as well as the role of feeding deterrents and attractants and other behavioural factors (Ananthakrishnan *et al* 1985; Ananthakrishnan 1986; Bell and Carde 1986).

Choosing hosts involves considerable selection pressure which varies with species which may be mono-, oligo or polyphagous, and wherever strict monophagy is ensured, selection may cause specificity to increase. Reduction in the suitability of less preferred species as compared to most preferred ones may also lead to increased host specificity. Alternatively a decrease in specificity would be favoured when it threatens larval survival, and by decreasing specificity females would tend to lay more eggs to decrease the risk of larval death, due to such factors as plant senescence (Rausher 1983). It is only after they choose to settle down on a substratum aided by these chemical cues that they commence feeding, deriving such substances as water, nitrogen, carbohydrates, lipids, proteins, amino acids and certain minerals, which are vital for growth, development and reproduction. Nutritional rewards may vary with plant tissues and insects discriminate between young, maturing and senescent tissues (Ananthakrishnan and Raman 1986).

3. Plant defense capabilities

Morphological and physiological traits such as leaf toughness, hairiness or trichomes, surface waxes, cell silicates and proliferation of wounded tissues, interfere with locomotion, feeding and oviposition. Colour may be a remote factor as they act at meaningful distances and prevent an insect from landing on a plant. Alongside colour, size, plant shape and density are other remote factors. Yellowish green leaves are mostly preferred i.e. that reflect light with wavelengths within 500–600 nm range. Due to deposition of additional lignin and cellulose, the plant tissues become more resistant to feeding action. Leaves of greater toughness cause increased larval mortality. Thick hypodermal layers have been considered a factor of resistance. Sometimes thick cell walls inhibit digestion and this is very well seen in the feeding of several grasshoppers. The recognition of C_4 plants with high photosynthetic capacity, and C_3 plants with low photosynthetic capacity reflects the differences in the nutritive value of plants and C_4 plants tend to be a poorer food

source for insects. Again solid stems are resistant to borer insects and hard, woody stems with closely packed, tough vascular bundles are the main resistant factors and in some cases the cortex also tends to be thick. Trichomes which are hair-like, cellular outgrowths of the epidermis of plants are among the more important morphological defenses of plants against insects. Some trichomes possess glands which exude chemicals which may be of the nature of a sticky film to trap insects. Their shape, size, density and erectness contribute to the degree of morphological resistance. The basic constituents of trichomes are cellulose and lignin and where they are very dense, biting insects have to consume them to reach the epidermis and in so doing death ensues due to an inadequate diet. Trichomes act as mechanical barriers to insect activity and insects are trapped or impaled by glandular, or non-glandular trichomes and more particularly by hooked trichomes (Juniper and Southwood 1986). Wounding results in the proliferation of cells and other plant products which act as defensive mechanisms. This takes us to the question of the nature of chemical defenses and implications of wounding in plants.

4. Implications of wounding in plants

Wounding disrupts the normal patterns of synthesis of chemical substances giving rise to quantitative and qualitative changes in terpenoids, some of which, after wounding, tend to inhibit the development of bacteria and fungi and are included in a class of phenolic antibiotic compounds termed phytoalexins of which benzoic acid, flavonoids and isoflavonoids are important. It must be recognized that steroids, alkaloids and phenylpropane compounds viz. lignins, tannins, phenols and quinones play an important role in the life of the plant. The rapid turnover rate of alkaloids, the involvement of steroids in plant hormone function, of the large array of polyphenols, flavonoids in wound reactions, point to the dynamic role of these substances. Phenylpropanes are formed through the shikimic acid pathway which serves for biosynthesis of phenylalanine and include also volatile aromatic compounds. Also involved are the isoprene units or C_5 compounds which in turn are constituents of a large number of terpenoids, mono, di- and sesquiterpenes discussed subsequently. Shikimic acid plays a fundamental role in the metabolism of aromatic amino acids and these are mobilized into the primary metabolism serving as storage chemicals or as regulation of biochemical process (Kogan 1977).

Wounding also results in increased synthesis of cinnamic acid derivatives, the most important being the accumulation of chlorogenic and caffeic acids. Chlorogenic acid is the mobile resource for the synthesis of other phenylpropane derivatives. The enzyme acting on phenylalanine is phenylammonialyase (PAL) and the production of chlorogenic acid in response to wounding is due to rise in PAL activity. Response to wounding in plants is also the formation of a large protective covering over the cut surface which may form a barrier against water loss and to various micro-organisms, and this is suberin or lignin. Lignin is a polyphenolic polymer, while suberin is a polymer of long chain fatty acids in which cinnamic or coumaric acids are associated. Free coumarin is released only upon cell disruption through the action of endogenous β -glucosidase. Wounding of plants through insect feeding also results in ethylene production which can influence a wide range of plant responses and alteration of plant growth, chlorophyll and foliar senescence. Feeding also promotes abscissic acid (ABA) levels which act as defenses for diverse plant stresses (Visscher 1983, 1987).

Signals are released from attacked sites or transported to uninfected or unwounded cells where they trigger almost immediately biochemical processes that produce chemicals which have primary roles in plant defenses. In some cases chemicals are produced at or near the sites of wounding to arrest the attacking pests. These chemicals are of the nature of insect-induced proteinase inhibitors and phytoalexin producers. An important role for plant cell wall fragments released at the wounding sites is envisaged. The majority of inhibitors from plants are specific for the serine class of proteins and protein inhibitors are widely distributed. Resistant varieties have doubled the level of trypsin inhibitors. Specificity and concentration are important aspects in proteinase inhibitors as plant defenses (Ryan 1983).

The large diversity of chemical substances results from plant metabolism and these phytochemicals involved in host plant resistance (HPR) are alkaloids, flavanoids, glycosides, terpenoids, tannins and lignins, which act as feeding deterrents, growth inhibitors, toxicants and oviposition deterrents. Recent researches also show that oviposition deterrents can offer the first line of defense against insects and active plant constituents cause the avoidance of egg-laying; besides chemical markers are left behind which prevent others from laying eggs at or near the site of already laid eggs. For many phytophagous insects selection of an oviposition site is a critical stage in host choice (Singer 1986). This is because newly hatched insects are incapable of selecting hosts till they have fed on the hosts selected by their mother.

Host plant selection is not merely an insect recognizing a particular chemical or avoiding it, but it is rather a process resulting from the integration of numerous chemical and nonchemical factors involving a subtle interplay of orientational, ovipositional and feeding factors as well as visual, tactile and environmental factors (Renwick 1989). Many of these non-nutritional factors which have no part to play in the metabolism of plants are termed secondary plant substances or allelochemicals.

5. Chemical defenses

Plants have provided thousands of compounds and with every passing day hundreds of them are being isolated and identified. Terpenoids, phenolics, proteinase inhibitors, nitrogen compounds, alkaloids and growth regulators including hormonomimetic compounds are known, which defend against insect attack (Kogan 1977). Terpenoids are biologically the most important class of natural plant products, acting as attractants for pollinators as well as feeding deterrents and toxicants. They are non-nitrogenous and use a 5-carbon-branched chain as a building unit. Pyrethrum from dried chrysanthemum is a toxic monoterpene, while gossypol of cotton, a sesquiterpene is a major defense chemical against cotton insects. Cucurbitacins are triterpenoids imparting a bitter taste and are feeding deterrents (only attractant to cucurbitaceous beetles). Azadirachtin from neem (*Azadirachta indica*) is a very effective monoterpene acting as a feeding deterrent at very low concentrations. Alkaloids are the best known among the toxins against insect attack, solanaceous hosts providing a host of examples like solanine, nicotine, tomatine, leptadine, strychnine, atropine and many of them are known to inhibit sugar receptors and hence block sugar responses. Phenolic compounds are non-nitrogenous compounds containing one or more hydroxyl groups attached to a

benzene ring. Tannins are polymeric phenolic compounds with strong protein adsorbing properties. Proanthocyanadins or condensed tannins elicit variable responses according to the concentration involved. Protein-complexing capacity of various biosynthetically limited polyphenols is the principal means by which polyphenols like tannins exert their defensive role in plants. This is particularly true of gallic acid which undergoes a wide range of modification, often oxidative, leading to a host of secondary metabolites (Brattsten and Sami Ahmed 1986). Proteinase inhibitors are polypeptides that bind to enzymes and are found in large quantities in seeds and foliage. Some plant species produce a variety of proteinase inhibitors, each with a different specificity. Although specific enzyme inhibitors and animal hormone analogues provide protection at lower concentrations, this is not the case in adapted insects even at higher concentrations. It is in this situation that plants produce novel substances, not present in attacked plants. In short, phenolic compounds which provide mechanical support for plants, also provide pre- and postinfestation factors involving polymeric phenols and phytoalexins which are allelopathic and allelochemic agents in insect-plant interactions. Secondary plant chemicals or allelochemicals, defined as the most ecologically active plant compounds, play a vital role in insect behaviour and are non-nutritional compounds which interfere with insect feeding and reproduction (Schoonhoven 1972). The array of allelochemical substances produced during shikimic acid metabolic pathway, serve as attractants, feeding deterrents, repellents and stimulants are implicated in host plant selection process involving host finding for feeding and oviposition by the adult female as well as host feeding for feeding, growth and development of the larva. Some of these are termed allomones favouring the producing organisms and others as kairomones, favouring the receiving organism (Feeny 1976; Rhoades and Cates 1976). Antibiotic effects of allomones relate to disruption of normal growth and development of larvae and reduced longevity and fecundity. Some allomones have antixenotic effects which disrupt normal host selection behaviour through acting as repellents, locomotory excitants and deterrents. Phenolic compounds are universally present in higher plants and possess one or more phenolic hydroxyl groups and as indicated earlier the phenylpropanes are important and formed in part via the shikimic acid pathway. They exist as monomers such as cinnamic acid, flavonoids, isoflavonoids and as polymers such as tannins and lignins. What is puzzling and complex is that it is not possible to describe insect and plant relationships based on phenolics alone, since different classes of secondary compounds may be involved at a number of different levels of interaction (Harborne 1978). Attractants more particularly to pollinating insects are mono and sesquiterpenes and volatile aromatic substances include simple aliphatic alcohols, ketones and esters. Some of the major plant volatiles attractive to insects are limonene, geraniol (mono), bisabolol (sesqui), vanillin, methyleugenol which are aromatic (Hedin *et al* 1971). Insects recognize odours from individual flowers limiting their attention to specific flowers. Having been attracted to flowers they depend on nectar for nutrition, especially the butterflies and it is noteworthy that increased amounts of nitrogen in nectar of flowers reflect the nutritional needs of the specific pollinators.

Recent attempts have shown the importance of tritrophic relationships on insect-plant interactions, which involve the host plant, the insect and the parasite or predator (Campbell and Duffey 1979; Barbosa *et al* 1982; Barbosa and Letourneau 1988). The discovery of tricosane and heptanoic acid as attractants of *Trichogramma*

and Potato tuber moth (Harborne 1978) has paved the way for further studies in this direction. There are also indications available of the role of volatiles emanating from the larval frass, larval cuticle and moth scales attracting the parasites towards the host organisms (Donald *et al* 1986). Interesting information is available of the secretions of dorsal and ventral abdominal glands of some coriids and pentatomids attracting parasites. The response of trichogrammatids and larval parasites to chemicals of the frass of *Heliothis armigera* and *Spodoptera litura* and antibiotic properties of such phenolic substances as resorcinol, phloroglucinol, gallic acid, pyrogallol to mention a few, are now being studied by the author and associates and appear to be of interest (Ananthkrishnan *et al* 1990).

That plants could change their chemical compounds to render their tissues less suitable for insect growth and reproduction as a direct result of the damage inflicted by insects has been well documented. At the same time the detoxification mechanisms of insects are equally well known, the major objective being the solubilization of a generally lipophilic compound into a water soluble one to be excreted, through the action of mono-oxygenases which are capable of oxidising a wide variety of oxidative reactions and referred to as MFOs are mixed function oxidases. Reduction of hydrolyses may also occur in the detoxification process. It must also be emphasized that while these processes are operative to counteract the defenses of plants and insects, phytophagous insects are equally capable of sequestering natural products regularly. Further, gall-forming species and plant-sucking insects tend to inject substances into the plant tissues that increase the nutritional quality, their saliva containing polyphenolic oxidative enzymes which tend to neutralize plant defense phenolic compounds and/or which could be involved in the production of auxins or auxin-like compounds (Miles 1972). In this connection the role of leaf miners appears to be of interest in that they retard senescence of leaves in which they feed to produce 'green islands' containing higher levels of cytokinins.

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Impact of natural products on the physiology of phytophagous insects

B P SAXENA and K TIKKU

Division of Insect Physiology, Regional Research Laboratory, Jammu Tawi 180 001, India

Abstract. The influence of naturally occurring toxic substances of plants such as alkaloids, rotenoids, antifeedants, growth inhibitors, hormone analogues, sterilants and antigonadial agents, on the physiology of some phytophagous insects is discussed.

Keywords. Natural products; insects; physiology.

1. Introduction

Insects have developed a variety of strategies in the guise of toxic molecules along with an appropriate delivery mechanism to face varied encounters in nature. The success of these natural weapons depend on the ways in which inbuilt mechanisms have been devised for synthesizing all sorts of molecules that are effective either on a specific target or act as general shoot out signals. Majority of these compounds act by interfering in the normal signalling mechanism of the nerve cells i.e. basically in the manner similar to the modern insecticides like the DDT and pyrethroids that interrupt the membrane permeability of cells due to an effect on bound proteins. Leads of similar nature have resulted in the industrial synthesis of many compounds that have their base in naturally occurring toxins like eserine, nereistoxin and pyrethrum etc. but unfortunately even after about 49 years of the discovery of DDT, out of about 200 synthetic insecticides discovered, the structure of only a few is based upon the clues provided by nature. The generally projected view is to discover the mode of action of natural products on pests including the insects so that synthesis is directed towards environmentally safe and commercially viable compounds that will lower down the populations of pests of agriculture and of vectors of diseases.

2. Toxic substances

2.1 Alkaloids and glycosides

These compounds have been found to exhibit some physiological action on the animals and are toxic to the man as well as to the insects. Though instances of alkaloids/glycosides being utilized or stored by some specific feeders like the Monarch butterfly (that stores cardiac glycosides to deter the predatory birds) (Roeske *et al* 1976) and *Cretonotos* moth (that utilizes the pyrrolizidine alkaloids of plants for synthesizing its pheromone) (Schneider *et al* 1982) are available but these compounds in general are very toxic to the insects and one of the first pesticides used by the man was the alkaloid nicotine from the tobacco plant *Nicotiana tabacum* L. A comprehensive review of this category of compounds has been published by Levinson (1976).

2.2 Pyrethrum

This insecticide belongs to the first generation of insecticides and was isolated from the plant *Chrysanthemum cinerariaefolium* (Casida 1973). Because of its property of a high toxicity against insects and negligible toxicity against the man and other warm blooded animals, it gained much popularity. However, due to its high degradation rate in the atmosphere especially in the presence of light, the use of pyrethrum was limited to the control of mainly the household pests. With the synthesis of pyrethrins and permethrin, more stability has been achieved in this group of compounds (Elliot 1977).

2.3 Rotenoids

These active components from *Derris* and *Tephrosia* are toxic to the insects and the fish. The active ingredients rotenone and deguelin are powerful insecticides but all species do not contain rotenoids (Marini Bettolo and Delle Monache 1975).

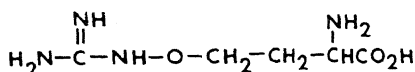
2.4 Ryanodine

This active principle from the woody portion of the south American shrub *Ryania* (family: Flacourtiaceae) is a complex substance (Wiesner *et al* 1967) used for the control of insects in the fields.

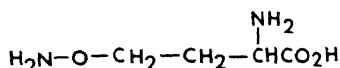
3. Antifeedants

These chemicals preventing insect attack belong to a large family of compounds most of which act specifically against specific insects and only a few have universal application. Many uncommon amino acids, found generally in seeds and preventing the attack of beetles and other insects (Bell 1977, 1980), non-hydrolysable tannins (flavanotannins) and cyanogenetic glycosides belong to the latter category. Such amino acids may also be present in the leaves and rhizomes of some plants.

Much work has been done on canavanine (2-amino-4-guanidinoxybutyric acid), the amino acid present in the seeds of several species of plants e.g. *Gymnocladus dioica* and *D. megacarpa*. The compound mimics arginine and is toxic to the insects and mammals on hydrolysis to L-canaline (figure 1) which acts as a neurotoxin. However, the bruchid beetle *Caryedes brasiliensis* can attack *Canavalia* seeds as its



L-Canavanine



L-Canaline

Figure 1.

enzyme system is capable of breaking canavaliine (Rosenthal *et al* 1978). Experimentally it has been shown that uncommon amino acids act as general antifeedants for *Locusta*, *Schistocerca gregaria* and *Chortoicetes terminifera* (Marini Bettolo 1983). Certain other feeding inhibitors from plants like *Parabenzoin tribolium*, *Clerodendron tricotomum*, *Orixa japonica* and *Angelica japonica* are known (Wada and Munakata 1971) while antifeedant activity has been seen in the oils of *Isocoma wrightii* and *Mentha pulegium* (Zalkow *et al* 1979). Some other plants having antifeedant properties worth mentioning are *Warburgia ugandensis* and *W. stuhlmannii* (Kubo and Ganjian 1981), *Artemisia capillaris* (Yano 1983), *Catharanthus roseus* (Meisner *et al* 1981), *Physalis*, *Withania* and *Nicandra* sps. (Ascher *et al* 1981), *Encelia* (Isman and Proksch 1985) and *Ajuga* sps. (Belles *et al* 1985). The steroidal alkaloid conessine from *Holarrhena antidysenterica* acts as an antifeedant for *Spodoptera litura* and *Pieris brassicae* (Saxena and Tikku 1988) while the alkaloids of *Adhatoda vasica* viz. vasicine, vasicinol, vasicinone, deoxyvasicine and deoxyvasicinone, deter feeding of *Aulacophora foveicollis* and *Epilachna vigintioctopunctata* (Saxena *et al* 1986). Azadirachtin from Indian neem tree *Azadirachta indica*, is a most versatile antifeedant and also exhibits many other physiological activities.

4. Growth inhibitors

Some terpenoids of sunflower e.g. kaurenoic and trachylobanoic acids inhibit larval growth (Elliger *et al* 1976) while others e.g. carvacrol, eugenol, farnesol and geraniol inhibit the embryonic development (Mehta 1979) of insects. Some of the other phytochemicals acting against the development of insects are chavicol (Ohigashi and Koshimizu 1976), two compounds from *Coleopsis lanceolata* (Nakajima and Kawazu 1980), C-glycosylflavones from *Zea mays* (Elliger *et al* 1980), certain norditerpene dilactones (Singh *et al* 1973) and sendanin from *Trichilia roka* (Kubo and Klocke 1982) etc. Conessine, from *H. antidysenterica* has larval growth inhibitory activity against *Aedes aegypti* (Saxena and Tikku 1988). The non-protein amino acid L-canavanine derails the growth and development of *Manduca sexta* (Dahlman 1977), *Musca domestica*, *M. autumnalis*, *Haematobia irritans* and *Stomoxys calcitrans* (Dahlman *et al* 1979) in addition of reducing the fecundity and fertility of *M. sexta* and effecting its nervous system (Kammer *et al* 1978; Palumbo and Dahlman 1978).

5. Hormone analogues

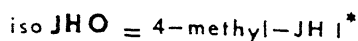
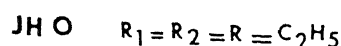
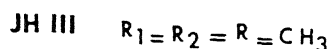
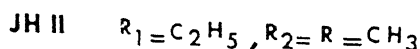
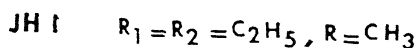
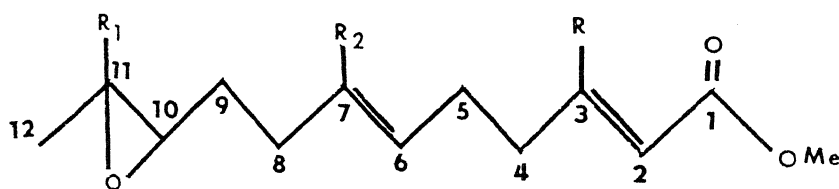
Till the discovery of the 'paper factor' (Slama and Williams 1966; Williams and Slama 1966) insect endocrinology was confined only to the academic interest. As the factor could produce permanently juvenile adults incapable of reproduction it was thought to make use of such compounds for insect control and Williams (1967) termed them as the 'third generation pesticides'. Because only the papers manufactured from the coniferous trees showed juvenile hormone (JH) activity, therefore the general belief at the time was that only gymnosperms possessed the JH mimicking substance [or the JH analogues (JHA)]. The very first report followed by a second one about such a type of activity being present in the

flowering plants was that of Saxena and Srivastava (1972, 1973) and this led to search for a trail of JHA existing in the flowering plants.

5.1 Evaluation and biological assay of JH/JHA

In order to term a compound as JHA it is very essential to know the true test for its evaluation. Primarily a JH exerts its effect on metamorphosis and a test compound must produce morphogenetic changes in the test insect while all other effects are of a secondary importance (Saxena *et al* 1978). Slama *et al* (1974) have described in detail the methodology that should be followed for a proper evaluation of such types of compounds and have calculated the ID/ED₅₀ values. For determining the effect on metamorphosis last instar nymphs or pupae should be subjected to the tests. The lesser important criteria for JHA tests are: (i) an inhibition of the embryonic development, (ii) stimulation of ovarian development and (iii) population assays.

The five natural JH identified so far are illustrated in figure 2. Numerous analogues of JH have also been synthesised by the private companies especially by the Zoecon Corporation of USA whose highly active patented products are the methoprene and hydroprene but on account of the development of resistance by the insects towards these JHA, the compounds could not find a successful place in the market. Moreover the slow action of such types of compounds could not stand before the rapid overnight control of the insect pests brought about by the pesticides. Such drawbacks have made the developed world lose interest in these analogues and now their use is recommended only in specific places like the hospitals etc for controlling pests like the pharaon ants since poisonous pesticide applications are avoided at such places.



* Relative & absolute configuration
at C-4 unknown

Figure 2.

6. Moulting hormones

These steroidal hormonal secretions of the prothoracic glands of insect larvae and of spongy cells of adult testes (Saxena and Tikku 1989) govern the insect moulting. A variety of ecdysterols are also present in the plants (Hikino and Hikino 1975) as these phytosterols can be easily synthesised by the plants by metabolism from the other steroidal sources (Levinson 1972). Reports are available on the presence of ecdysones in the pteridophytes (6 families, 44 sps.), the gymnosperms (6 families, 26 sps.), and the angiosperms (22 families, 46 sps.) (Jacobson and Crosby 1971).

7. Antijvenile hormones

This new category of compounds has been placed under the class of the fourth generation pesticides. Bowers *et al* (1976) found two naturally occurring chromene-derivatives in *Ageratum houstonianum* that could produce precocious adults (i.e. adults moulted directly from the fourth instar nymphs by deleting the formation of the fifth instars during the development process) and also effected the ovaries of such adults. These derivatives were termed as Precocene I and Precocene II (figure 3) and in the insect body these compounds are broken down resulting into the

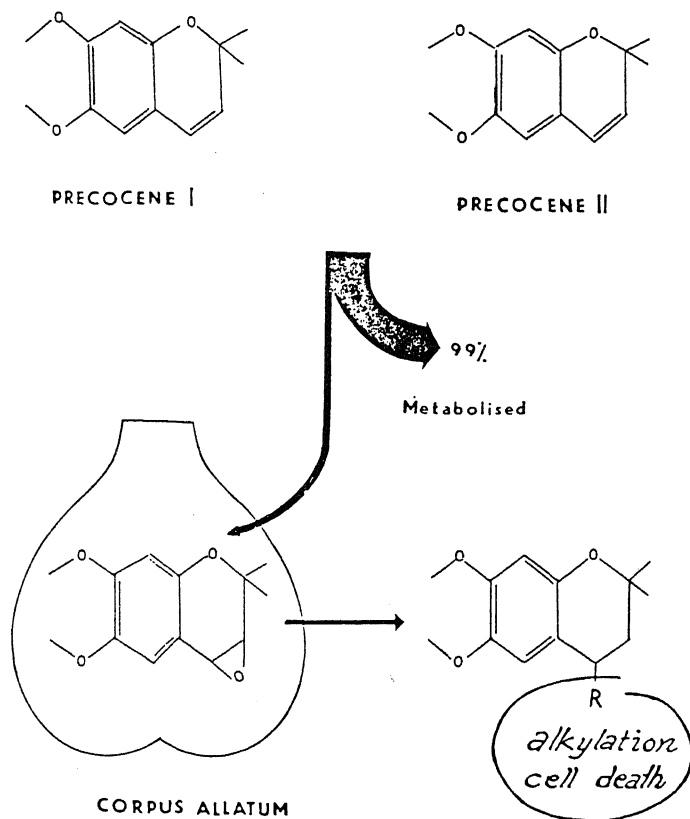


Figure 3.

production of another compound (3,4-epoxide) that reacts with the proteins in the corpus allatum leading to their destruction (Bowers and Martinez-Pardo 1977) and thereby to an impaired JH synthesis. The low titres of JH in the nymphs or the larvae result into their transformation into adults.

8. Sterilants

The fecundity and fertility of the insects can be disturbed in multiple ways e.g. either by a direct effect on the germ cells or by an indirect hormonal disruption, whether of neurosecretion or of the juvenile or the moulting hormones. The classical chemosterilants are alkylating agents bringing about sterility by their property of effecting the DNA and thereby the germ cells while the sterilants differ from such alkylating agents in lowering down the rates of fecundity and fertility but not causing any harm to the germ cells. Such sterilants are exemplified by compounds like conessine, several alkaloids of *A. vasica*, aristolochic acid, and plumbagin (Saxena *et al* 1979, 1986; Saxena and Tikku 1988; Thappa *et al* 1988).

8.1 Conessine

This steroidal alkaloid from *H. antidysenterica* sterilizes *Dysdercus koenigii* (Thappa *et al* 1988) when given in drinking water (doses used: 5 and 10 ppm) for 72 h and untreated mates provided to the treated individuals. In 5 ppm the most remarkable action of the compound was being more effective in inducing sterility in the untreated females crossed with treated males rather than showing a more strong action against the females that were given the alkaloid in feed but with the increase of dose to 10 ppm the fecundity of treated females was also reduced and the laid eggs were sterile for treated individuals of both the sexes (table 1). The effect on both fecundity and fertility of normal females crossed with treated males indicates the transfer of some factor from the affected males into the female genital system that reduces the number of eggs and makes such eggs sterile while conversely the normal sperm of the untreated male provided to the alkaloid-fed female perhaps is not able to fertilize its eggs due to a changed physiological environment of the female tract that does not provide an environment conducive for normal fertilization and fertile eggs are not formed in this way.

8.2 Alkaloids of *A. vasica*

The fecundity and fertility of *D. koenigii* was adversely affected by 5 tested alkaloids

Table 1. The percentage reduction in laying and hatching of eggs of adult *D. koenigii* affected by conessine.

	Dose (ppm)	1st batch		2nd batch		3rd batch	
		Laying	Hatching	Laying	Hatching	Laying	Hatching
Untreated ♀ treated ♂	5	18.69	00.0	23.95	2.2	31.79	27.8
	10	20.96	00.0	50.38	1.5	66.87	0.5
Treated ♀ untreated ♂	5	00.00	45.6	00.00	14.8	33.94	31.9
	10	25.68	00.0	19.86	00.0	66.35	0.8
Control		00.00	100	00.00	100	07.60	100

of *A. vasica* (Saxena *et al* 1986) given for 72 h in drinking water (dose: 0.1 and 0.3%). The alkaloids were vasicine, vasicinol, vasicinone, deoxyvasicine and deoxyvasicinone (table 2). Vasicinol displayed the maximum and deoxyvasicinone the minimum activity. The female *D. koenigii* adult lays its normal number of eggs whether mated or not (unpublished results) but a collapse of eggs (within 2–3 days of oviposition) of untreated females paired with vasicinol treated males having sperms of normal appearance indicates some disturbance in the chorion formation of such eggs which leads to a slight blackening in their colour followed by crumpling. In the case of vasicinol treated females the lowering in the number of laid eggs is apparently related to a blocking of the oviduct by oocytes which inhibits the descent of the vitellar oocytes and their resorption causes the death of such females. All other allelochemicals of *Adhatoda* bring about a reduction in the oocyte numbers. The *Adhatoda* alkaloids are also effective in reduction of the progeny of *Tribolium castaneum* in doses of 0.1 and 0.5%.

8.3 Aristolochic acid-I

This product from *Aristolochia bracteata* in addition of acting as a sterilant for *D. koenigii*, *Ae. aegypti* and *T. castaneum*, also interferes in the ecdysis (Saxena *et al* 1979). The high dosage of 0.002% totally prevents egg laying in the adults moulted from treated 5th instar nymphs of *D. koenigii*, the next dose i.e. 0.001% is again totally inhibitory for females while comparatively the male treatments are not as effective though hatching percentage becomes highly reduced in the eggs, the lowest dose of 0.0005% does not cause any reduction in the first batch of eggs but in the

Table 2. Cumulative fecundity and fertility of 3 batches of eggs in *D. koenigii* adults treated with *Adhatoda* alkaloids at 0.1 and 0.3% level (source: Saxena *et al* 1986).

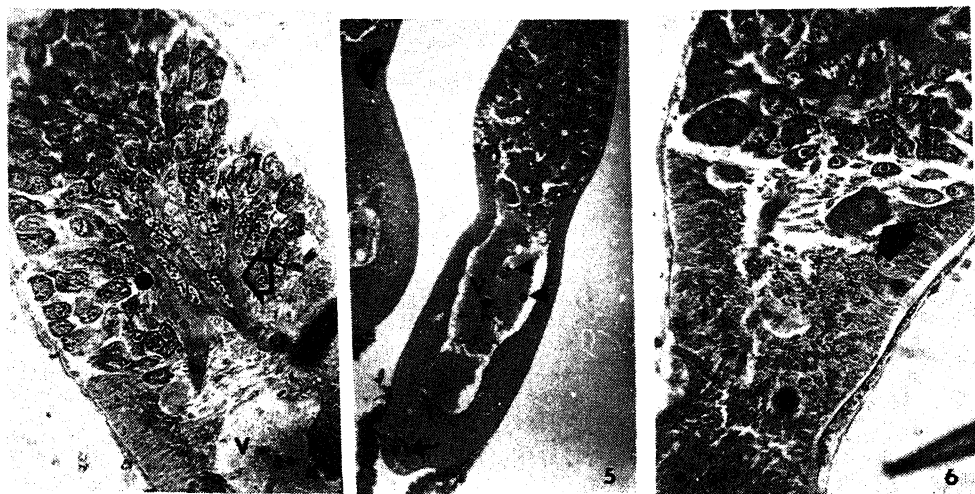
Treatment	Sex cross			Average fecundity (no. \pm SE)		Average fertility (%)		Sterility index	
	♂	×	♀	0.1%	0.3%	0.1%	0.3%	0.1%	0.3%
Vasicine-HCl	T	×	T	74.9 \pm 6.9	64.3 \pm 6.4	60.3	67.9	64.0	65.0
	T	×	U	93.1 \pm 6.4	90.7 \pm 10.0	80.0	67.0	40.4	51.2
	U	×	T	101.5 \pm 7.0	72.8 \pm 5.0	77.3	72.0	37.0	58.0
Vasicinol	T	×	T	56.3 \pm 6.6	46.2 \pm 3.0	49.1	30.5	77.8	88.7
	T	×	U	40.0 \pm 5.8	42.3 \pm 3.1	37.7	20.4	87.8	96.7
	U	×	T	53.5 \pm 7.5	53.8 \pm 4.0	49.8	45.0	78.6	80.7
Deoxyvasicine	T	×	T	102.2 \pm 6.3	91.4 \pm 7.0	65.3	63.3	46.4	53.6
	T	×	U	96.7 \pm 9.9	93.7 \pm 6.5	54.1	63.1	58.0	52.5
	U	×	T	94.7 \pm 7.0	85.1 \pm 6.3	61.3	53.0	53.3	63.8
Vasicinone-HCl	T	×	T	94.7 \pm 6.4	36.8 \pm 4.3	58.9	40.7	55.2	88.0
	T	×	U	91.0 \pm 7.4	104.3 \pm 8.0	64.1	56.7	53.2	52.5
	U	×	T	88.3 \pm 7.0	84.5 \pm 5.7	51.2	43.0	63.7	70.9
Deoxyvasicinone-HCl	T	×	T	100.5 \pm 6.2	104.3 \pm 8.8	90.8	100.0	26.7	18.6
	T	×	U	99.2 \pm 7.3	111.0 \pm 6.8	90.5	98.0	27.9	15.2
	U	×	T	120.3 \pm 9.2	108.0 \pm 8.0	89.8	92.5	13.2	22.1
Control	U	×	U	127.5 \pm 6.2	130.9 \pm 9.0	97.7	98.0		

T, Treated; U, untreated.

subsequent second and third batch the fecundity and fertility goes on declining (70 and 30 eggs respectively in 2nd and 3rd batch in comparison with 110 eggs of the controls). In the case of adult treatments the effect was not as marked as in the treatments to 5th instars though the hatching capabilities get significantly reduced. While lower doses are not effectively able to control the vitellogenesis, though the manner in which yolk is deposited seems very haphazard and the number of oocytes is reduced, the higher doses prevent maturation of ovaries of both the adult treatments and of the adults moulted from 5th instar nymphs. However all the doses affected acutely the ovaries of adults formed from treated 4th instars as such ovaries remained very small consisting of almost only the tropharium. The vitellar portion was undeveloped and ovaries retained their larval yellow covering. Figures 4-6 show a large number of trophocytes and their nutritive cords but only a few oocytes in the tropharium. Sometimes the undifferentiated vitellar portion contained 2-3 oocytes in addition to the corpus luteum and the oviduct, indicating an imbalance in the hormonal system governing the maturation of the oocytes or at the cystocyte stage of the ovarian development. Such a type of effect is totally different from that of the classical chemosterilants that directly influence the DNA of the cells. Impairment of vitellogenesis was also observed in the ovaries of *Ae. aegypti* (doses used: 0.001-0.00025%) and that of *T. castaneum* (doses used: 1-10 ppm).

8.4 Plumbagin

This nephthoquinone reduces the fecundity of the red cotton bugs (Joshi *et al* 1989), affects spermatogenesis (Saxena and Tikku 1988) and causes sterility in the houseflies (unpublished results). Low doses of 1-2 μg of plumbagin brought about a



Figures 4-6. Affected ovary of *D. koenigii*. 4. Tropharium with trophocytes (T) and nutritive cords (arrow). The vitellarium (V) is empty. 5. Juvenile ovary with empty vitellarium (V) and corpus luteum (arrow). Arrow-head points to the oocytes. 6. Magnified view of lower portion of tropharium showing a single oocyte (arrow).

delay of the ovarian development of adult bugs but higher doses disrupted vitellogenesis (Joshi *et al* 1989). The experiments on male houseflies (unpublished results) have revealed that treatments at adult stages were very effective (table 3) as compared to those given to the wandering larvae.

According to Joshi and Sehnaal (1989), plumbagin inhibits or delays the imaginal ecdysis and oviduct metamorphosis in *Dysdercus cingulatus* and an injection of makisterone A reverses the effect of the compound. They found that plumbagin affects the prothoracic gland and the pericardial cells which in turn suppresses the biosynthesis of ecdysteroids. In *in vitro* experiments it was shown by them that the presence of plumbagin in culture media could restrain the ecdysteroid biosynthesis and it was concluded that plumbagin exerts a direct action on the prothoracic gland. According to other reports plumbagin inhibits chitin synthetase activity (Kubo *et al* 1983) and interferes in ecdysone biosynthesis due to its inhibitory action on the enzyme ecdysone 20-monooxygenase (Mitchell and Smith 1988). Our experiments have shown that the compound affects the spongy tissue of the testis of *D. koenigii* (Saxena and Tikku 1988) whose secretion is steroidal in nature (Saxena and Tikku 1989). The disintegration of the cells of the spongy tissue indicates that the testis is not provided with the requisite quantities of steroids required for an uninterrupted spermiogenesis. Also, the appearance of vacuoles in spermatocytes and a reduction in the number of axonemal microtubules of the sperms of *D. koenigii* (Saxena and Tikku 1988) points out to the fact that plumbagin does not specifically act on the prothoracic gland and inhibits biosynthesis of ecdysteroids but such an effect is a generalised one on cells including the glandular ones.

9. Antigonadal agent

The term 'antigonadal' is applied for those compounds that act particularly against the gonads i.e. their action is neither mediated by the endocrine system of the insect nor do they behave as alkylating agents. β -asarone is one such compound (Saxena *et al* 1977), isolated from the oil of *Acorus calamus* L. that brings about a regression of the ovaries of *D. koenigii* by its antigonadal action. The oil in vapour form also sterilizes *Thermobia domestica*, *M. domestica*, the females and males of *D. koenigii* and a number of stored product insects (Saxena and Rohdendorf 1974; Mathur and Saxena 1975; Saxena and Mathur 1976; Saxena *et al* 1976; Koul *et al* 1977).

Products of similar nature that are detrimental for the survival of innumerable pests of the insect kingdom are stored in the existing flora in unlimited quantities and tapping of such sources is the urgent need of the hour. The essential role played

Table 3. Effect of plumbagin treatment on the egg laying and hatching of adult males of *M. domestica* crossed with untreated females.

Dose (2 μ l/fly)	Reduction in egg laying (%)	Hatching (%)
0.05%	76	0
0.01%	71	0
0.005%	64	0

by the plant is to supplement the insect thriving on it with the nutrients required for its growth and development. In addition, while providing almost all the precursors for the constituents of some very important hormones and the pheromones, the plants concomitantly ensure their own existence against the voracious feeders by synthesizing certain chemicals like the tannins to reduce the intensity of the attacks because tannins are known to be indigestible. Some other compounds like azadirachtin, gossypol and canavanine are also utilized by the plants for strengthening their defences. The saponin gossypol is a growth retardant, decreases larval survival, causes pupal deaths and also increases the time taken for pupation (Dongre and Rahalkar 1980). This important pest controlling agent is found in the leaf glands of cotton plants and in the enthusiasm for increasing the yields, the cultivated varieties became glandless and prone to infestation. Azadirachtins' antifeedant action is just one of the multifaceted aspects of this phytochemical that is most effective in disrupting growth, acting as a sterilant and inhibiting the insect moulting. In locusts the compound deters feeding even if the azadirachtin-sensitive chemoreceptors on the mouthparts are bypassed by directly injecting it into the haemolymph which indicates its effect on the passage of food through the gut and the speculations are that the azadirachtin-poisoned gut is unable to expand sufficiently for a successful moulting (Mordue (Luntz) *et al* 1985). Azadirachtin also lowers down the ecdysteroid titres and according to the findings of Pener *et al* (1988) the compound affects ecdysteroid metabolism and/or prothoracicotropic hormone release. Canavanine's toxic and sterilant action also acts as a safeguard for the host plants.

The role played by the moulting hormone in an insect's life is well known because the insect is totally dependent upon phytosterols for the synthesis of the hormone. The physiology and reproduction is governed by ecdysteroids but the major drawback that limits their desirable exploitation is the difficulty of penetration of sterols through the insect cuticle. Therefore till the stage is reached when absorption of these vital plant products through the gut or the body is possible, the ecdysteroids have a limited scope for use as insect controlling agents.

The isoprene units of terpenoids are available in plants but so far it is not known whether the phytophagous insect gets any precursor from the host plant for the production of JH, the most important physiological secretion of the corpus allatum. The synthesized analogues are also losing ground due to the preferences for fast acting compounds and, because of the development of resistance by some economically important insects (Brown and Hooper 1979), a potent compound like the methoprene has also become of a limited use. Precocenes which appeared to be physiologically very important compounds in acting as antijuvenile hormone agents have receded in the background on account of their cytotoxicity.

The other hormones including the neurosecretion are indigenous products of the insect body and till now no link has been established with their relation to the insects' feeding activity.

Pheromones, having a direct bearing on the modulation of the insect behaviour, are comprised of widely unrelated groups like the aldehydes, ketones, alcohols etc. and these groups are the constituents of a number of phytochemicals that the insect gets from its host plants but whether the compounds have a decisive participation in the synthesis of the volatile product is not yet known. However the work of Schneider *et al* (1982) has shown that totally unrelated compounds like the

pyrrolizidine alkaloids are used in pheromone production and more work needs to be done along this direction to explore the co-relation between natural products and synthesis of pheromones by the insects as this area has an immense use in future.

The sterilitant chemicals from plants truly appear to be the answer for the physiological agents that are in demand due to their property of causing a disharmony in the hormonal balance of the pest. Compounds like conessine, *Adhatoda* alkaloids, aristolochic acid, and plumbagin are the products of plant origin and sterilize male as well as female insects. These phytochemicals are applied in very low dosages and their detailed mode of action at cellular or hormonal level needs to be probed further. The antigonadal property of the active *cis*-form of β -asarone has given a clue for the synthesis of similar compounds with the appropriate positions of O-methyl groups so that only compounds exhibiting activity of similar nature are obtained rather than toxic or antifeedant compounds (Saxena *et al* 1977).

Now-a-days work on genetic engineering and DNA recombination techniques are believed to be the answer for future control measures in the light of the toxicities caused by *Bacillus thuringiensis* in the insect pest but recent work has revealed that mosquito larvae have already overcome the toxic effects of *B. sphaericus* (Aly *et al* 1989) and if one pest has developed detoxification mechanism the possibility of other such organisms following suit is always there. JHA protagonists also at one time were putting forward the argument that as these compounds were structurally similar to those produced by the insect body, so chances of development of resistance were the least but what happened was contrary to the expectations. Similarly if mosquito larvae are able to digest the *Bacillus* poison and the trend goes on increasing, genetically engineered plants will lose their efficacy. The other aspect of the problem that needs a thoughtful approach is that, presently the *B. thuringiensis* incorporated tobacco plants are considered a safe bet against the tobacco hornworm but tomorrow if similar methodologies are adopted to protect other crops, will not the toxins have a disastrous accumulative effect on those living beings (including mammals) who would regularly consume the edible parts. Therefore toxicological studies need to be conducted in advance before commercialization of the process.

Our stronghold against the pests can only be maintained by a proper screening of the plants and the evaluation of their products on the physiology of the insect. If the activity of the phytochemical is of a desirable nature, constraints should be followed in its overexploitation so that the problems of resistance that are being encountered for the synthetic analogues of pyrethrins (Scott and Georgiou 1985) are not faced and at the same time no harm should come to the predators and the parasites. An ideal approach would be to put into use the aggregated effect of varied properties of the natural products e.g. the toxic, antifeedant, growth retardant, and sterilitant etc. and advocate their proper application in integrated control schemes so that the pest species are effectively brought below the level of nuisance.

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Digestive physiology and food utilization of the larvae of *Earias vittella* (Lepidoptera: Noctuidae) from its malvaceous host plant

S S KRISHNA

Department of Zoology, University of Gorakhpur, Gorakhpur 273 009, India

Abstract. The hydrogen-ion concentration of the contents of fore-, mid- and hindgut of the larva of *Earias vittella* (F.) lays within a pH range of 8.8 and 9.6. These caterpillars possessed the ability to break down starch, raffinose, maltose, melibiose, sucrose and proteins. Synthesis of certain higher oligosaccharides indicating transglycosidic activity was also noticed during hydrolysis of raffinose, melibiose and sucrose in *in vitro* experiments. The activity of carbohydrases detected in the larval midgut was variably influenced by the hydrogen-ion concentration of the medium. Further, they differed from one another in their relative strength. Amylase was the most powerful and α -galactosidase was the weakest of all. An *in vivo* examination into the fate of fed starch, maltose, cellobiose and lactose within the gut revealed the physiological competency of the caterpillar to utilise only the first two carbohydrates (the former partly and the latter completely) through swiftly operating processes of digestion and absorption.

Movement of ingested food through the gut of the caterpillar was a rapid process. The first lot of food residue passed out of the hindgut within a mean time of 103 min after feeding. However, the entire alimentary canal got cleared of the ingested food only 36 h after a meal.

Keywords. *Earias vittella*; larva; gut physiology; food utilisation.

1. Introduction

The spotted bollworm, *Earias vittella* (F.) (= *Earias fabia* Stoll) is a major pest of cotton and okra in the tropics (Butani and Jotwani 1984). Physiological competency of this pest, in terms of hydrogen-ion concentration, distribution, nature and characteristics of digestive enzymes, rate of movement of ingested food and metabolic transformations of some fed sugars within the gut of the caterpillar (the main damaging stage in the life of this insect), to utilise the various dietary constituents obtained while feeding on its above mentioned natural host plants has already been reported (Krishna and Pandey 1974; Krishna and Tripathi 1986; Tripathi and Krishna 1988). A reiteration of some of these findings in this communication together with unpublished information (Tripathi 1985) concerning (i) the appearance of higher saccharides during the hydrolysis of certain sugars under *in vitro* conditions in the larval midgut and (ii) the *in vivo* fate of certain carbohydrates in the caterpillar was considered appropriate for a meaningful comprehension of insect-plant relations, from nutritional physiology point of view, between this pest and its food plants.

2. Materials and methods

Third instar (5 days old) caterpillars of *E. vittella* reared on tender seeds of okra (Vishwapremi and Krishna 1974) were used for all tests. Before using them in the

various experiments, these larvae were allowed access to feed only on moist filter paper for a period of at least 24 h, placed inside glass containers (35 × 100 mm) covered at the top by muslin fastened by elastic bands. Such pre-experimental treatment of the larva was found essential to clear its alimentary canal from all previously ingested food obtained from okra seeds.

2.1 *pH measurement of the gut*

The hydrogen-ion concentration of the different regions of the alimentary canal was determined by the 'range-indicator method' (Waterhouse 1940) after feeding the caterpillars for 10 min, on crushed okra seeds blended separately with 7 indicators (bromophenol blue, bromocresol green, bromocresol purple, bromothymol blue, phenol red, cresol red and thymol blue). A 20:1 ratio was maintained in the proportion of seed and dye components in all the mixtures. Such preparations also helped in finding out the pH range of okra seed (food of the larvae).

2.2 *Distribution, nature and characteristics of digestive enzymes*

This part of the study first involved *in vitro* monitoring of the presence of different digestive enzymes in the lumen of the caterpillar's midgut followed by determination of the influence of hydrogen-ion concentration on the activity of the detected carbohydrases and estimating their relative strength as well.

2.2a *Qualitative survey of digestive enzymes:* For an examination of this aspect, the caterpillars were first fed on glucose mixed with bromophenol blue (sugar-dye proportion maintained as before) for 5 min (for the purpose of providing adequate stimulus for proper amount of enzyme secretion) and then removed from food. Contents of the lumen of the midgut from each of 10 such fed larvae were extracted following the procedure of Krishna (1955, 1958) and pooled together. Later, 0.1 ml aliquot of this extract was incubated at 37°C for 24 h with similar volumes of (i) a suitable substrate, (ii) one of 4 arbitrarily chosen buffers having pH values 5.8, 6.8, 7.2 (McIlvaine 1921) and 9 (Oser 1971) and, where necessary, (iii) an activator. A drop of toluene was added to this mixture to prevent growth of microorganisms. The enzymes tested for and substrates employed are listed in table 1. After incubation, in case of carbohydrases, products of digestion and/or the presence of undigested substrate, if any, in the mixtures at different pH values were detected using appropriate biochemical tests or by adopting the technique of paper chromatography (Krishna 1958). Assessment of proteinase activity was based on the methodology initially implemented by Saxena and Bhatnagar (1958) and subsequently carried out by Krishna and Saxena (1962). Activities of polypeptidase and lipase were determined as described by Krishna (1955). Each enzyme experiment was repeated thrice and identical results were obtained. All trials were accompanied by properly set controls wherein the same quantity of distilled water replaced midgut extracts in each incubated mixture.

An interesting feature observed during the determination of activity of carbohydrases hydrolysing melibiose, raffinose and sucrose was the appearance, in every assay, on the chromatogram of a new sugar (in addition to the normal products of digestion of these carbohydrates), the R_g value (ratio of distance

Table 1. List of enzymes tested for and the substrates employed.

Enzyme	Substrate
Carbohydrases	
Amylase	Soluble starch, 0.3% solution
Invertase	Sucrose, 5% solution
Maltase	
(α -glucosidase)	Maltose, 5% solution
α -fructofuranase	Raffinose, 5% solution
α -galactosidase	Melibiose, 5% solution
β -glucosidase	Cellobiose, 5% solution
β -galactosidase	Lactose, 5% solution
Proteinase	Gelatin on photographic plate
Polypeptidase	Peptone, 5% solution
Lipase	Olive oil emulsion

migrated by the new sugar spot to that migrated by glucose spot on the chromatogram) of which showed great variation amongst them. It was of interest to ascertain the time of appearance and relative concentration (based on visual comparison of colour intensity of the spots on the chromatogram) of these unidentified sugars in the reaction mixtures kept for incubation at 37°C with one or the other of the 3 above mentioned carbohydrates. For this purpose, a separate series of experiments was arranged along the lines similar to that already described with the difference that the incubation mixture set up in all these tests was always maintained at pH 9. At the end of every hour of incubation up to a period of 7 h 30 μ l sample was drawn from each reaction mixture and the sugars present in it, including the new saccharide, were resolved by paper chromatography as reported earlier.

2.2b Influence of hydrogen-ion concentration on the activity of carbohydrases: This part of the investigation first entailed the constitution of a series consisting of 6 samples of the midgut extracts of the caterpillar, prepared as outlined already. For determination of amylolytic activity, the volume of extract maintained in each sample was 0.2 ml. The reaction of the extract in these samples was adjusted to 6 different values of hydrogen-ion concentration by adding to each 0.2 ml of a suitable buffer formulated as mentioned earlier and ranging from 5.8–9.5. A control series comprising two samples, wherein an identical volume of distilled water was kept in place of gut extract, was also set up. One of these samples was similarly buffered to pH 5.8 while in the other the pH was regulated to 9.5. After adding 0.1 ml of 1% sodium chloride solution (serving as an activator) followed by 0.2 ml of 0.3% starch solution and two drops of toluene to each sample of both series, the mixtures were incubated at 37°C for 18 h. The reaction was then stopped by the addition of 0.15 ml of N-hydrochloric acid to each mixture. Each sample was then treated with 0.075 ml of potassium iodide-iodine reagent (Smith and Roe 1949) and the solution in it was finally diluted with distilled water to 5 ml.

Blue colour would develop in solutions, the intensity of which would depend upon the concentration of undigested starch in the different incubated mixtures. This concentration was measured colorimetrically (Smith and Roe 1949) using an

appropriate standard for eventual determination of per cent hydrolysis of starch at different pH values.

To estimate the activity of remaining carbohydrases, the volume of midgut extracts held in each of the 6 samples of the series was 0.1 ml. Controls were arranged as before to form a separate series. pH conditions in the samples of both these series were variously maintained, like in amylase experiment, by the addition of 0.1 ml of one of the prepared buffers. Solutions in each of these samples subsequently received 0.1 ml of a concerned substrate (5% solutions of maltose, melibiose, raffinose or sucrose) and two drops of toluene prior to keeping them for incubation at 37°C for 18 h. The rest of the methodology relating to paper chromatography, elution and colorimetry adopted for quantitative evaluation of the pH effect on each carbohydrase activity was basically akin to that described by Krishna (1958).

2.2c Relative strength of different carbohydrases: Consideration of this issue involved division of the pooled larval midgut extracts into 5 lots of 0.1 ml each. Every lot was then supplemented with 0.1 ml of a buffer of pH 9 [rationale for the choice of such pH value was to approximate the hydrogen-ion concentration of the experimental medium to that which naturally prevailed within the midgut of the caterpillar, as observed by Krishna and Tripathi (1986) and reported in this communication as well], 0.1 ml of an appropriate carbohydrate substrate of known concentration (0.3% in the case of starch and 5% with respect to other 4 sugars listed in the previous section) and, in tests relating to assessment of the strength of amylase activity, 0.1 ml of 1% sodium chloride solution to function as an enzyme activator. Two drops of toluene were then added to each sample and the mixtures were incubated at 37°C for 24 h. At the end of incubation, the reaction mixture containing starch was treated with 0.15 ml of N-hydrochloric acid to inhibit enzymatic activity of amylase. The follow-up procedure to determine the strength of amylase in the larval midgut, based on colorimetric estimation of hydrolysis of starch, was similar to that already described.

In the case of other incubated mixtures, the termination of incubation first led to the drawing out, from every sample, of two fractions of 0.02 ml each for eventual subjection to paper chromatography (Krishna 1958). The loci on the base line of chromatogram, where these fractions were applied, had been earlier treated with 0.007 ml of 0.1 N sodium hydroxide solution. This would ensure stopping of enzymatic reaction as soon as the incubated mixture was applied to the chromatogram. Concentration of the undigested substrate in each incubated mixture was subsequently determined by elution and colorimetry (Krishna 1958). Comparison of the degree of hydrolysis of the different substrates would indicate the relative strength of the carbohydrases.

2.3 *Passage of food through the gut*

For monitoring the movement of ingested food through the larval digestive tract, an individual was initially fed for 2 min on okra seed diet mixed with bromophenol blue. Dissections of such fed larvae were performed at different time intervals after the meal so as to determine the position of the dye in the various parts of the gut. This technique would apparently indicate the locus of ingested food in relation to a point of time, provided the dye behaves as a marker and its transport through the

larval gut synchronises with that of the food mixed with it. Time taken for the arrival of first coloured excreta after food intake was also recorded. For this purpose, each fed caterpillar, freed from adhering food particles, was transferred to a clean rectangular glass container ($45 \times 45 \times 15$ mm) having a glass top through which the insect was constantly observed for ejection of its first coloured faeces. Difference between the time of ingestion of the last meal and the evacuation of the first coloured excreta would indicate the rate of passage of the first lot of eaten food through the gut. The total time required for all the ingested food to pass through the alimentary canal of the caterpillar was also calculated in a similar manner. The entire course of experiment was repeated at least 5 times employing a fresh larva for each replicate.

2.4 *In vivo* fate of certain carbohydrates

In order to ascertain the time and site of digestion and absorption of certain individual nutrients *in vivo*, the fate of starch, maltose, cellobiose and lactose was followed along the lines as reported in a similar study involving fructose, sucrose and raffinose in this species (Krishna and Pandey 1974). However, in the present series of tests, the caterpillars were allowed to feed for 30 min on one of these carbohydrates mixed with bromophenol blue as per proportions already stated and samples of haemolymph and midgut contents from these fed larvae were collected at 30, 60, 90 and 120 min after a meal while that of excreta 6–8 h after nutrient intake for chromatographic analysis.

3. Results

3.1 *Hydrogen-ion concentration*

The hydrogen-ion concentration of all the 3 regions of the gut of *E. vittella* larva was moderately alkaline and lay within a range of pH 8.8–9.6 (table 2) despite the caterpillar feeding on okra seeds having a strong acidic property (pH 4.6–5).

3.2 *Digestive enzymes*

The presence or absence of various digestive enzymes, based on an *in vitro* investigation and determined qualitatively at 4 randomly chosen pH values, in the

Table 2. pH in different regions of the digestive tract of *E. vittella* larva.

Indicator	Foregut	Midgut	Hindgut
Bromophenol blue	> 4.6	> 4.6	> 4.6
Bromocresol green	> 5.2	> 5.2	> 5.2
Bromocresol purple	> 6.8	> 6.8	> 6.8
Bromothymol blue	> 7.6	> 7.6	> 7.6
Phenol red	> 8.4	> 8.4	> 8.4
Cresol red	> 8.8	> 8.8	> 8.8
Thymol blue	> 8.4 < 9.6	> 8.4 < 9.6	> 8.4 < 9.6
Range of pH	> 8.8 < 9.6	> 8.8 < 9.6	> 8.8 < 9.6

larval midgut of this noctuid species is given in table 3. Only 5 carbohydrases (amylase, invertase, maltase (= α -glucosidase), α -fructofuranase and α -galactosidase) and a proteinase were found to occur in the midgut of this caterpillar. Interestingly, the activity of α -galactosidase, α -fructofuranase and invertase, in addition to causing hydrolysis of their corresponding substrates, also led to the production of variable types of oligosaccharides of unknown composition (table 4). Although all these unidentified sugars made their appearance within the first hour itself of digestion of melibiose, raffinose and sucrose respectively, their relative concentration, however, varied with the passage of time during the breakdown of these substrates.

3.2a Influence of hydrogen-ion concentration on the activity of carbohydrases: The degree of activity of the various carbohydrases occurring within the midgut of the larva of *E. vittella* varied with the hydrogen-ion concentration of the medium (table 5). Amylase activity was very high exceeding 90% hydrolysis of starch at all tested pH values save 9.5 and reached a maximum at pH 7 or 8. Amongst the remaining carbohydrases, only invertase showed consistently more than 50% activity at different pH values peaking at pH 8 which was slightly higher than that recorded at pH 9. Maltase and α -galactosidase exhibited a similarity in having their highest level of activity at pH 6.4 while α -fructofuranase attained its zenith in bringing about hydrolysis only at pH 9.

3.2b Relative strength of digestive carbohydrases: The foregoing account clearly reveals that under the influence of any one common pH value different carbohydrases detected from the midgut of the larva of *E. vittella* possess varying capacities to hydrolyse their corresponding substrates. This indicates the existence of an obvious disparity in the relative strength of these carbohydrases. This also implies that greater the strength of a particular enzyme, the more will be the efficiency of the insect to digest the corresponding substrate. In other words, the degree of utilisation of different carbohydrates by this larva depends, to some extent, on the relative strength of the appropriate enzyme. With a view to check this

Table 3. Distribution of digestive enzymes in the midgut of the larva of *E. vittella* (*in vitro* study).

Enzyme	pH values			
	5.8	6.8	7.2	9.0
Carbohydrases				
Amylase	+	+	+	+
Maltase (= α -glucosidase)	+	+	+	+
Invertase	+	+	+	+
α -fructofuranase	+	+	+	+
α -galactosidase	+	+	+	+
β -galactosidase	-	-	-	-
β -glucosidase	-	-	-	-
Proteinase	+	+	+	+
Polypeptidase	-	-	-	-
Lipase	-	-	-	-

+, Indicates presence of enzyme; -, indicates absence of enzyme.

Table 4. Relative concentration of oligosaccharides synthesised, their mean R_f value (ratio of distance migrated by an oligosaccharide spot to that migrated by glucose spot on the chromatogram) and break-up of other hydrolytic products detected during the course of activity, within a stipulated time frame, of α -galactosidase, α -fructofuranase and invertase at pH 9 from the midgut of *E. vittella* larva (*in vitro* study).

Enzyme (substrate)	Duration (h) of hydrolysis of the substrate, products detected and relative concentration (denoted by increasing number of plus signs) of the oligosaccharide (O) formed							Mean R_f value* of oligosaccharide
	1	2	3	4	5	6	7	
α -galactosidase (melibiose)	mb ga g O (+)	mb ga g O (++)	mb ga g O (++)	mb ga g O (+++)	mb ga g O (+++)	mb ga g O (++)	mb ga g O (+)	0.07
α -fructofuranase (raffinose)	r mb ga g f O (+)	r mb ga g f O (+)	r mb ga g f O (++)	r mb ga g f O (+++)	r mb ga g f O (++)	r mb ga g f O (+)	r mb ga g f O (+)	0.18
Invertase (sucrose)	s f g O (++)	s f g O (++)	s f g O (+++)	s f g O (+++)	s f g O (++)	s f g O (+)	s f g O (+)	0.38

*Based on 8 determinations.

f, Fructose; g, glucose; ga, galactose; mb, melibiose; r, raffinose; s, sucrose.

Table 5. Estimates of per cent hydrolysis of various carbohydrates at different selected pH values by corresponding carbohydrases from the midgut of *E. vittella* larva.

Carbohydrase	Substrate	Per cent hydrolysis (rounded to the nearest integer) of substrate at different selected pH values					
		5.8	6.4	7.0	8.0	9.0	9.5
Amylase	Starch	92	93	97	97	93	72
Invertase	Sucrose	51	67	52	70	69	61
Maltase (= α -glucosidase)	Maltose	22	62	58	51	30	0
α -fructofuranase	Raffinose	15	18	26	22	40	20
α -galactosidase	Melibiose	24	27	17	12	11	0

point and to obtain a clear confirmation of this phenomenon in the caterpillar's digestive physiology, the activities of 5 carbohydrases described above have been quantitatively assayed at one common pH value over an extended period of experimental time.

Amongst the different carbohydrases, amylase was the strongest since almost the entire substrate (99.2%) was hydrolysed. Next in order came invertase and α -fructofuranase which hydrolysed 72.5 and 50.5% respectively of their corresponding substrates. Maltase was a moderately active enzyme and degraded 40.5% of the disaccharide maltose while α -galactosidase was the weakest of the carbohydrases capable of breaking down only 15% of melibiose to its monosaccharide components.

3.3 Movement of food through the gut

The ingested food passed quite rapidly through the larval foregut and accumulated in its midgut. Within 6–10 min of feeding, the first lot of food residue began to enter into the hindgut where it stayed for a period of 70–122 min before its eventual defaecation. In the midgut, food, nonetheless, continued to remain even beyond 16 h. However, by the end of 24 h, there was hardly any food noticed in this region. The entire alimentary canal of the caterpillar, however, got cleared of all the ingested diet within a mean time of 36 h after a meal.

3.4 *In vivo* analysis of certain carbohydrates

Although observations procured from *in vitro* analysis of the distribution of various carbohydrases in the midgut of the larva of *E. vittella* described above, suggest the ability of this insect to utilise the corresponding substrates, they do not provide clue concerning the actual time of digestion of all these nutrients and subsequent absorption of products yielded from such digestion within the caterpillar's midgut. In order to acquire more information on this aspect, the fate of starch, maltose, cellobiose and lactose ingested by this insect has been traced.

Table 6 summarises the findings relating to the occurrence of one or the other of the aforementioned ingested carbohydrates in the midgut, haemolymph and excreta of the caterpillar and their subsequent biochemical transformations within the former two tissues of the insect body. Within 30 min of a meal, all the 4 eaten

Table 6. Carbohydrates in the midgut (M), haemolymph (H) and excreta of *E. vittella* larva at different time intervals after ingestion of starch, maltose, cellobiose or lactose (*in vivo* study).

Carbohydrate	Time interval (min)								Excreta (collected 6–8 h after feeding)
	30		60		90		120		
	H	M	H	M	H	M	H	M	
Starch		st		st		st		st	st
		m	m	m	m	m	m	m	
	g	g	g	g	g	g	g	g	
Maltose		m	m	m	m	m	m	m	
	g	g	g	g	g	g	g	g	
Cellobiose		c		c		c		c	c
Lactose		l		l		l		l	l

c, Cellobiose; g, glucose; l, lactose; m, maltose; st, starch.

saccharides were detected in the midgut, although starch and maltose during this period had undergone partial hydrolysis to yield small amounts of products of their digestion. At the same time, some glucose began to show up in the haemolymph of caterpillars fed on either of these two carbohydrates. As the time interval after ingestion of starch or maltose increased to 60 min, the latter sugar also, interestingly, started to appear in the larval haemolymph. Neither cellobiose nor lactose could be digested by this caterpillar in its ventricular region at any time during the entire 2 h experimental period. Chromatographic analysis of the larval excreta collected even as late as 6–8 h after the insect feeding on each of these two carbohydrates further showed that the food residue contained cellobiose and lactose. Curiously enough, some undigested starch was also detected in such faecal matter.

4. Discussion

This investigation highlights several interesting features in the digestive physiology of *E. vittella* larva which warrant elucidation in the light of whatever information is available on similar issues on other phytophagous insects, more importantly with reference to different species of noctuids.

The high pH values having identical range in all the 3 regions of the gut of this caterpillar are quite at variance with those reported earlier for the same insect (Srivastava and Mathur 1966). The adoption of indicator paper touch method by these authors, where contamination of the paper by the surrounding haemolymph was an inevitable drawback, had presumably seriously affected the proper determinations of the hydrogen-ion concentration of this insect's digestive system in their studies. It must also be mentioned here that the enhanced pH value recorded in the midgut in this study fits in with the generally prevalent more alkaline nature of the contents of the gut in phytophagous insects (Wigglesworth 1984). The absence of any change in the gut pH, despite the caterpillar feeding on okra seeds possessing strong acidic nature, evidently shows the existence of a powerful buffering mechanism within the larval gut as in a majority of other insects (House 1974).

As in the midgut of caterpillars of two other noctuids, *Spodoptera litura* and *Trichoplusia ni* (Mathur 1966), *E. vittella* larva also exhibited the activity of amylase, invertase, maltase and a proteinase in its ventricular region and thus possessed the capacity to digest starch, sucrose, maltose and proteins. However, the spotted bollworm differed from *S. litura* and *T. ni* in not being endowed with the ability to hydrolyse lactose or lipids in view of the absence of β -galactosidase and lipase. The competency of this caterpillar to digest melibiose observed in the present investigation contradicts the earlier report of Krishna and Pandey (1974) on the non-existence of α -galactosidase enzyme in this larva. One possible reason for the failure of these authors to detect the presence of this enzyme (the weakest of 5 carbohydrases recorded in the larval midgut) is their adoption of an indirect method of testing for the occurrence of this enzyme through raffinose hydrolysis (instead of using melibiose, a more appropriate substrate). The presence of cellobiose and lactose in the excreta discharged by caterpillars fed on these sugars coupled with the absence of β -glucosidase and β -galactosidase in the larval midgut (as noticed in *in vitro* tests) confirms the total inefficiency of this insect to utilize

these sugars, both of which do not occur in the tender seeds of okra or cotton (natural host plants of this insect) (Mehta and Saxena 1973; Mani *et al* 1980). The appearance of some starch, despite its hydrolysis within the insect's midgut by a very powerful amylase, in the faeces of caterpillars ingesting this polysaccharide endorses the observations of Mehta and Saxena (1973) pointing to the existence of a limitation in the utilisation of this carbohydrate by these individuals. Presumably, amylase serves as a useful adjunct in the larval gut enzymes profile to provide the sugar requirements of the insect through partial digestion of starch, whenever obtained through feeding either from natural malvaceous food plant cotton or from certain experimentally tested non-malvaceous crops like *Zea mays* and *Pisum sativum* (Mehta and Saxena 1973) all of which, perhaps, contained some chemical ingredients restraining the activity of this carbohydrase *in vivo*. The occurrence of appreciably high levels of invertase and α -fructofuranase activity would obviously be of great value to the larva to properly utilise the sucrose present in its foods (Mehta and Saxena 1973; Mani *et al* 1980) and raffinose (determined only in *P. sativum*) (Mehta and Saxena 1973) eaten by it. Since maltose or melibiose, as such, was not present at all in the diets used in experimental studies with this insect (Mehta and Saxena 1973; Mani *et al* 1980), the enzyme maltase might be coming into action subsequent to the production of maltose derived from hydrolysis of starch by amylase and the role of α -galactosidase might be to breakdown melibiose following its yield from the digestion of raffinose by α -fructofuranase. This partly explains for the presence of these enzymes at very low strength. Correlation of the influence of hydrogen-ion concentration on the activity of different detected carbohydrases with pH range prevalent within the midgut of the larva of *E. vittella* clearly indicates that the lumen of this region in the alimentary canal of this caterpillar is most favourable only for the activity of invertase and α -fructofuranase although considerable digestion of starch is also possible.

Results on the study of fate of certain carbohydrates within the midgut of this larva clearly revealed that digestion of starch and maltose occurred during the first half hour after ingestion. Appearance of glucose at the same time in the larval haemolymph indicated a simultaneous absorption of the product of digestion of these two carbohydrates from the midgut. The absorption of some ingested maltose as such into the blood of the larva 1 h after a meal is, indeed, interesting and, in this respect, it not only resembled another disaccharide sucrose (Krishna and Pandey 1974) but also served as one more experimental evidence in support of the statement 'occurrence of sugars in insect haemolymph is also consistent with relatively non-specific absorption' (Wyatt 1967).

An important factor that affects digestion and absorption of food materials in insects, in general, is the period of stay of these substances in the digestive and absorptive portions of the gut of these animals. The longer the duration of halt of a food constituent in these areas, the greater are the chances of its complete digestion and subsequent absorption. In *E. vittella* larva, although the first lot of ingested food that diffused in the midgut took only a mean time of 7.7 min to enter the hindgut, the overall period of stay of the diet was considerably longer in the ventricular region and always extended beyond 16 h since intake of a meal. This was, undoubtedly, much greater than that reported for food movement in *S. litura* and *T. ni* (Mathur 1967). The prolonged duration of retention of food in the larval midgut of *E. vittella* coupled with the ability of this caterpillar to swiftly digest the

fed carbohydrates and absorb their hydrolytic products (Krishna and Pandey 1974 and present data) strongly suggest that these physiological characteristics of the gut of this insect are well established to ensure maximum utilisation of these carbohydrates.

The presence of a new unidentified oligosaccharide, besides appearance of hydrolytic products, in midgut in *in vitro* tests involving raffinose, melibiose or sucrose confirms transglycosidic activity, a biochemical phenomenon excellently manifested in many phytophagous insects (Waterhouse 1957; Saxena and Bhatnagar 1961; Saxena and Krishna 1963; Krishna and Pandey 1974; House 1974; Srivastava and Krishna 1977), of α -fructofuranase, α -galactosidase and invertase in spotted bollworm caterpillar. The fact that the unknown higher saccharides synthesised in the course of degradation of these sugars were all not the same (based on their R_g values) provides a reasonable basis to suggest the existence, in *E. vittella* larva, of varying patterns of transglycosidic action which, however, has a relationship with the substrate on which the carbohydrase reacts initially.

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Aspects of insect resistance in crop plants

S JAYARAJ and S UTHAMASAMY

Department of Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, India

Abstract. Importance of growing insect resistant crop in pest management programmes is discussed with reference to some of the major aspects of insect resistance in crop plants in Tamil Nadu and emphasis has been made in highlighting the fact that the plant resistance to insect should form the foundation upon which other components of the integrated pest management should be built.

Keywords. Resistant crops; antixenosis; antibiosis; tolerance; induced resistance; transgenic plants; genetics of resistance; biotype; pest management.

1. Introduction

Plant resistance to insects encompasses the application of principles of insect-plant interactions to pest management. Resistance of a plant to an insect is defined as the 'relative amount of heritable qualities possessed by the plant which influences the ultimate degree of damage done by the insect' (Painter 1968). Plant resistance as an approach in pest management offers many advantages. Crop varieties that are resistant provide an inherent control which involves no expense nor environmental pollution problems and is generally compatible with other methods of insect control. Insect resistant varieties are more valuable where crops are low in value particularly in developing countries, and also in situations where lack of technical knowledge limit the proper use of costly insecticides. Growing insect resistant crops is now highly valued in pest management programmes. Depending on the level of resistance, it can be used either as the principal method or as a supplement to other measures of pest management. It also serves as a safeguard against the release of varieties which may be more susceptible than the existing ones. The major aspects of insect resistance in crop plants and the achievements in Tamil Nadu are discussed in this paper.

2. Resistance—general aspects

Resistance or susceptibility of plants to insects involves a cause and effect relationship between the insects response(s) to the plant and, in turn, the plants reaction(s) to the insect; for example, lack of attraction of insects to a particular plant for oviposition or feeding and the unsuitability of plants for insects.

Painter (1968) classified varietal resistance into 3 categories:

- (i) Non-preference: When a plant possesses characteristics that make it unattractive to insect-pests for oviposition, feeding or shelter.
- (ii) Antibiosis: When the host plant adversely affects the bionomics of the insects feeding on it.
- (iii) Tolerance: When the damage to the host plant is only marginal despite its

supporting an insect population of a size sufficient to damage severely susceptible hosts.

The term antixenosis was suggested to replace non-preference by Kogan and Ortman (1978). A plant's resistance may be caused by one or more of these factors. Antibiosis is considered a major aspect of resistance; under some circumstances, even non-preference can be equally important, particularly where small infestations cause severe plant damage. One example of the latter is feeding by stem borers which cause dead hearts and/or white heads and insect transmission of plant diseases. Under field conditions, non-preferred crop varieties frequently escape infestations and when insects are caged on non-preferred hosts, they lay fewer eggs and smaller populations develop. Both antibiosis and non-preference affect insect populations. Tolerant varieties do not inhibit insect populations and it takes longer time for build up of populations. In this the insects are exposed more to their natural enemies. In recent times, tolerant varieties are considered to be a valuable component in pest management programmes.

3. Causes of resistance

The non-preference, antibiosis and tolerance mechanisms of resistance result from a series of interactions between insects and plants. These interactions are governed by the plant characters viz. morphological (biophysical) and physiological and/or biochemical.

3.1 Morphological characters

Many morphological characters contribute to the resistance of plants to insect pests. These include trichomes, surface waxes, hardness of plant tissues, thickening of cell walls and cuticle, rapid proliferation of tissues, anatomical modifications of plant organs, colour and shape of plant parts. Some morphological structures additionally contain allomones that affect the behaviour and metabolism of phytophagous insects. The role of plant morphological characters in insect resistance was reviewed by David and Easwaramoorthy (1988). The role of important characters in imparting resistance in crop plants are as follows:

3.1a Trichomes: Plant trichomes are of several kinds and have physiological and ecological functions. The trichomes are glandular hairs and are abundant on the leaves of many species of plants. The pubescence may affect locomotion, attachment, shelter, feeding, digestion and oviposition of insects e.g. cotton leafhopper, *Amrasca devastans* (Dist.) on bhendi (Uthamasamy 1985) and green peach aphid, *Myzus persicae* on *Solanum* species (Gibson 1971). Mechanical effects of pubescence depend on density, erectness, length and shape of trichomes (Norris and Kogan 1980). In addition, glandular trichomes exude secondary plant metabolites which may interfere with locomotion. The role of pubescence in host plant resistance has been reviewed by Webster (1975) and Norris and Kogan (1980).

Non-glandular and/or glandular trichomes entrap, immobilize or impale many pests. The first instar larvae of the cotton pink bollworm, *Pectinophora gossypiella* Saunders hatching from eggs laid on the vegetative parts of cotton plants are

disoriented by trichomes and hence, the number of larvae reaching the bolls is reduced (Smith *et al* 1975; Wilson and Wilson 1977). Laboratory observations on the movement of neonate larvae of *Heliothis virescens* (F.) on upper and lower leaf surfaces and petioles of 4 cotton varieties indicate that pubescence provides a mechanism of resistance to such movement (Ramalho *et al* 1984).

Pubescent plants are not generally preferred by small herbivores as it is difficult for their feeding organs to reach the site of feeding. Hairiness in cotton is associated with its resistance to the leafhopper, *A. devastans* (Sikka *et al* 1966). Trichomes interfere more with the feeding of nymphs than of adults of *A. devastans* (Uthamasamy 1985). Resistant varieties have a greater number of long trichomes on the midrib and lamina than fewer and shorter trichomes on susceptible bhendi varieties. Laminar trichomes without adequate length or long trichomes without adequate density are not effective in providing resistance in bhendi and cotton (Uthamasamy 1985; Balraj Singh and Atwal 1976).

The high degree of resistance in *Solanum mammosum* L. to *Aphis gossypii* G. and *Henosepilachna vigintioctopunctata* (F.) is attributed to the high density, length and erect disposition of hairs on the leaves (Sambandam *et al* 1972).

Leaf trichomes interfere with the oviposition of *A. devastans* on cotton (Agarwal and Krishnananda 1976; Teli and Dalaya 1981) and on bhendi (Uthamasamy 1986). Effective hair length on the ventral surface of midveins showed a significant negative correlation with the number of eggs laid (Khan and Agarwal 1984). Lukefahr *et al* (1975) found that a cotton strain with a glabrous character reduced the *Heliothis* population by 68%. On the other hand, Mehta (1971) has observed that the spotted bollworm, *Earias vittella* (F.) prefers *Gossypium hirsutum* leaves over bolls for oviposition in view of high density of hairs on the leaves. Agarwal and Katiyar (1974) have recorded the lowest oviposition and damage by *E. fabia* in growing points in smooth-leaved cotton varieties compared to medium and highly pubescent varieties. Pubescent cotton types supported more eggs of *E. vittella* than the glabrous Deltapine smooth leaf, which indicates the non-preference for a glabrous surface for oviposition (Sellammal Murugesan 1982). Sharma and Agarwal (1983) have observed that the leaf hairiness correlated significantly and positively with the number of eggs laid by *E. vittella* under both field and laboratory conditions.

3.1b Surface waxes: Plant waxes have the primary function of maintaining the water balance but they also interfere with insect-plant relationship either positively or negatively. In castor, no bloom and single bloom varieties have a relatively low population of the castor semilooper *Achaea janata* L. compared to varieties with double and triple blooms. So also, the resistance to the leafhopper, *Empoasca flavescens* (F.) has been found to be associated with the waxy blooming (Jayaraj 1976). The resistance to the mite, *Tetranychus telarius* Koch in castor has also been associated with the leaf bloom (Chandrasekaran *et al* 1964).

3.1c Hardness: Hardness of plant tissues is associated with resistance in insects. In sorghum, the resistance to the shootfly *Atherigona varia soccata* Rondani is imparted due to the presence of thick cells with distinct lignification (Blum 1968) and also hardness of leaf sheaths (Singh and Jotwani 1980). Proliferation of tissues and production of gummy exudates in cotton is associated with the resistance to the

stem weevil *Pempherulus affinis* Faust. Moco cotton (*G. hirsutum* race Marie Glands) arrests the development of grubs by producing a gummy exudate (Dharmarajulu *et al* 1948). The resistance in MCU 3 cotton variety is due to the drowning and death of grubs in the gummy exudate compared to the susceptible MCU 5 in which the grubs develop normally (Parameswaran and Chelliah 1985).

Stem thickness has been reported to be associated with the resistance to the sugarcane borer *Diatraea saccharalis* (Fabricius) (Agarwal 1969). The denticles on the midrib of leaves, the number of vascular bundles, the lignification of cell walls and the sclerenchymatous tissues play a role in the resistance to first and second instars. Sugarcane varieties with a hard stem are less susceptible to the internode borer *Chilo sacchariphagus indicus* (Agarwal 1969; David 1979). Rind thickness coupled with high fibre content are important factors in resistance to the sugarcane shoot borer *Chilo infuscatellus* Snell (Rao 1962, 1967). Sugarcane varieties with loose fitting leaf sheaths are relatively less susceptible to mealy bugs (Sithanantham 1973; Mehta *et al* 1981) and scale insects and eriophyid mites (Agarwal 1969). In sorghum, varieties with tightly wrapped leaves around the stem are resistant to the bug *Peregrinus maidis* Ashmead while the susceptible varieties have loosely attached leaves (Agarwal *et al* 1978).

3.2 Chemical factors

Insect resistance in plants is also due to the chemical constituents which may be either qualitative or quantitative. These chemicals occur within certain parts of the plant or in specific stages of plant growth. The herbivores' behaviour and adaptation to the host plant are influenced by the chemical composition of the host plant. These chemicals may be physiological inhibitors and/or nutritional deficiencies. A resume of biochemical bases of resistance in crop plants of both hereditary and non-hereditary categories and their usefulness in pest management was reviewed by Jayaraj *et al* (1988). These chemicals are known to influence the non-preference and antibiosis mechanisms.

3.2a Feeding stimulants

These are secondary plant substances and have no primary function in plants. However, some compounds such as sugars and amino acids stimulate the feeding activity in insects. The resistance in rice to the brown plant hopper *Nilaparvata lugens* (Stal) is attributed to lower contents of aspartic acid, asparagine, valine, alanine and glutamic acid which act as phagostimulants. An increase in amino acids and proteins and a decrease in mineral contents in susceptible varieties of castor and bhendi infested by the leafhoppers *Empoasca flavescens* (F.) and *A. devastans* were reported by Jayaraj (1967) and Uthamasamy *et al* (1971).

3.2b Other compounds: In rice, oxalic acid acts as a feeding deterrent. A higher concentration of oxalic acid in 'Mudgo' inhibits the feeding by BPH (Saxena 1986). Rice varieties resistant to the striped stem borer *Chilo suppressalis* (Walker) contain an ovipositional deterrent, while susceptible varieties contain some ovipositional attractants. Spraying extracts from these plants on rice varieties altered the

ovipositional behaviour of the borer (Pathak and Dale 1983). Terpenes, alcohols, esters, acids and some phenols are known to act as attractants and/or repellants and these also play a role in imparting resistance to insects in crop plants.

3.2c Toxic chemicals in host plants: Chemicals such as alkaloids, phenolics, flavonoids, and terpenoids are reported to be toxic to the insects and so influence the biology of the insects. In rice, the resistance to gall midge *Orseolia oryzae* (Wood-Mason) is mainly due to antibiosis. More free amino acids and phenols, but less sugar in the shoot tip of resistant varieties versus susceptible varieties apparently explain this phenomenon (Peraiah and Roy 1979). Oryzanone is reportedly a larval attractant and benzoic and silicic acid are larval growth inhibitors in rice plants. High nitrogen content and a greater percentage of starch purportedly account for the susceptibility of rice to the yellow stem borer *Scirpophaga incertulas* (Walker). Antibiosis in 'Taichung 16' was attributed to biochemical factors (Saxena 1986). The resistance to TKM 6 plants to the striped stem borer *C. suppressalis* is due to the production of certain allomones. Increased silica content in resistant rice varieties affects the mandibular development in the rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Hanifa 1971). Low carbohydrates leading to a narrow C:N ratio and a low calcium content in insecticide treated plots of rice were found to attract higher numbers of the blue leafhopper *Zygina maculifrons* (Mani and Jayaraj 1976).

In cotton, gossypol and tannins affect the susceptibility and oviposition behaviour of *E. vittella* (Sharma and Agarwal 1983). Gossypol decreases the nutritional quality of bolls by forming complexes with amino acids, proteins and enzymes. Some factors other than gossypol and tannins are also reported to protect cotton plants from bollworm (Raman and Ananthakrishnan 1986).

3.3 Plant nutrition and resistance

The host nutrition, physiological status of the host plant as well as quantitative differences in the nutritional components can influence the resistance or susceptibility to insects. It is reported that rice varieties resistant to brown planthopper contain less of asparagine compared to susceptible varieties (Sogawa and Pathak 1970). The asparagine content in rice plants is influenced by the nitrogenous fertilizer applied.

In cotton, more of total amino acids in the stem weevil susceptible MCU 5 than in the resistant MCU 3 and Deltapine 45 was reported by Parameswaran and Chelliah (1985). Obviously, the resistant varieties did not satisfy the nutritional requirements of the weevil. The feeding response of the tobacco caterpillar *Spodoptera litura* (F.) was more towards sucrose than other sugars. Sucrose was the most favoured sugar and galactose and glucose the least preferred ones (Parasuraman and Jayaraj 1983). In sorghum, varieties with high silica content were found to be resistant to the shootfly *A. v. soccata* (Ponnaiah 1951). The white-backed planthopper *Sogatella furcifera* (Horvath) resistant rice varieties ARC 10550 and IET 6123 contained more sugars and less amino nitrogen compared to the susceptible T(N)1 (Gunathilagaraj 1983).

In brinjal, the spotted beetle *Epilachna vigintioctopunctata* resistant accessions possessed low quantities of amino acids compared to the susceptible accessions

(Natarajan 1971; Sambandam *et al* 1972). Lower quantities of amino acids were found in the resistant *Cucumis callosus* (Rotte) Cogn. compared to cultivated muskmelons susceptible to the gourd fruit fly *Dacus cucurbitae* Coq. (Chelliah and Sambandam 1971). The red pumpkin beetle *Raphidopalpa foveicollis* preferred cucurbit plants with more nitrogen and protein and low carbohydrate and phenols (Raman and Annadurai 1985).

The resistance to the leafhopper *E. flavescens* in castor is influenced by the nutritional status of the varieties. The concentration of sucrose, glucose and fructose was high in the resistant varieties while only sucrose and glucose were observed in the susceptible and in very low amounts in tolerant varieties. The resistant varieties were not preferred by the leafhopper because of their nutritional inferiority as revealed by the low nitrogen, free amino acid and peptide contents (Jayaraj 1967). Accumulation of total carbohydrates and low levels of total nitrogen resulting in a wider sugar/nitrogen ratio were responsible for the non-preference in bhendi varieties resistant to the leafhopper (Uthamasamy *et al* 1971).

4. Genetics of resistance

A knowledge of plant genetics relative to insect resistance is essential to understand the basic aspects of resistance. This can contribute to the development of crop varieties with resistance which may reduce the development of insect biotypes adapted to the resistant varieties. In addition, knowledge on plant genetics can be applied to the development of isogenic lines which are used to identify the mechanisms of resistance as well as multi line plant populations etc. Resistance to many of the pests of crops is of polygenic nature and so imparts only a moderate level of resistance. Not much has been studied on the inheritance of this type of resistance in crop plants. Most of the high level of resistance known are monogenic. The generation of information on the number and type of genes involved in insect resistance depend on the thoroughness with which the germplasm is screened.

Detailed studies on the genetics of resistance in rice to brown planthopper, green leafhopper, white backed planthopper and gallmidge have been made and the number of genes that govern the resistance have been identified (table 1). Similar studies have been conducted in wheat, sorghum, maize and cotton. However, the precise role played by individual genes has not been studied so far in many crops.

5. Development of insect resistant crop varieties

The insect resistant variety should form the basis of IPM on which other methods are built-up. The role of insect resistant varieties in plant protection in Tamil Nadu was reviewed by Jayaraj (1976). During the two decades through intensive research and resistance breeding programmes, the Tamil Nadu Agricultural University has developed many crop varieties resistant to key pests (table 2). The usefulness of these varieties in pest management programmes has been brought to light (Jayaraj *et al* 1988). Rice varieties resistant to particular BPH biotype and cultivars with different resistant genes have been developed for effective management of the pest. The rotational use of resistant varieties with known genes could make the varieties stable in the field by slowing down the process of biotype development (Chelliah

Table 1. Genes governing resistance in rice to pests.

Gene	Rice accession	Reference
Brown planthopper		
Bph1	Mudgo, Co 22, MTU 15	Chelliah (1985a)
bph2	ASD 7	
bph3	Rathu Heenati	
bph4	Babawee	
bph5	ARC 10550	Khush <i>et al</i> (1985)
Bph6	Swarnalatha	Kabir and Khush (1988)
bph7	T 12	---
bph8	China Saba, Col 5. Thailand	Unpublished results
Bph9	Pokkali Balamawee	---
New dominant gene (undesig-nated)	ASD 11, T 7, V. P. Samba	Bharathi (1989)
White backed planthopper		
Wbph1	N 22	Chelliah (1985a)
Wbph2	ARC 10239	
Wbph3	ADR 52	
Wbph4	Podiwi-A8	
Wbph5	N'Diang Marie	Wu and Khush (1985)
Green leafhopper		
Glh1	Pankari 203	Chelliah (1985a)
Glh2	ASD 7	
Glh3	IR 8	
glh4	PTB 8	
Glh5	ASD 8	
Glh6	TADL 796	
Glh7	Moddai Karuppan	
Gall midge		
Gm1	Usha, Samridhi, Bd 6-1	Chaudhary <i>et al</i> (1985)
Gm2	Surekha, IET 6286	---
Zigzag leafhopper		
Zlh1	Rathu Heenati	Angeles <i>et al</i> (1985)
Zlh2	PTB 21	
Zlh3	PTB 33	

and Bharathi 1985). The development of TKM 6 with multiple resistance to several rice pests has been a significant achievement and this variety has been used as a donor in the development of many national and international varieties currently under cultivation.

In sorghum varieties resistant to the shootfly, stem borer and grain midge, leafhopper, whitefly and stem weevil resistant varieties in cotton, pod borer resistant cultivars in pulses, leafhopper resistant varieties in bhendi are a few of the crop varieties that have been developed at this University. In addition, the University also collaborates with many national and international institutes in identifying the sources of donors, evaluation of breeding lines and testing them in hot-spots.

Table 2. Insect resistant crop varieties in Tamil Nadu.

Insect	Crop and variety	Level of resistance
	Rice	
Brown planthopper	Co 25, ASD 5 Co 42, ADT 36, PY 3, ADT 37	Resistant
Green leafhopper	TKM 6, ASD 5 Co 25, ADT 37 ADT 38 PY 3	Resistant Moderately resistant
Yellow stem borer	TKM 6	Resistant
Gall midge	TKM 6, MDU 3, GEB 24	Resistant
Leaf folder	TKM 6, GEB 234, TKM 2	Resistant
	Cotton	
Stem weevil	MCU 3	Resistant
Leafhopper	MCU 5, MCU 9	Resistant
Whitefly	LPS 141, LK 861, Supriya	Resistant
Bollworms	Abhadita	Resistant
	Sorghum	
Shootfly	Co 1	Resistant
Rice Weevil	Co 4, Co 18, Co 19	Resistant
	Sugarcane	
Scale	Co 8014, Co 617, Co 6501	Resistant
Internode borer	Co 283	Resistant
Shoot borer	Co 281, Co 6601	Resistant
	Brinjal	
Aphid	Annamalai	Resistant
	Jasmine	
Eripolyrid mite	Pari Mullai	Resistant

Resistance breeding both by conventional methods and new innovative techniques are gaining momentum and many new lines are in pipe-line. The model adopted in developing an insect resistant variety in rice is depicted in figure 1.

6. Induced resistance

This is a non-heritable resistance wherein the host-plants are induced to impart resistance to tide over the pest infestation. This is possible by manipulation of fertilizer application, chelates, biofertilizers, organic amendments etc. Such manipulations also bring about changes in biochemicals of the host-plants. Application of higher doses of potassium confers resistance to some pests. Decreased infestation of leaf folder, thrips, brown planthopper, green leafhopper and stem borer has been reported in rice (Chelliah 1985b). Among the chelates, Zn-EDTA complex has been reported to induce resistance in rice to the leaf folder and

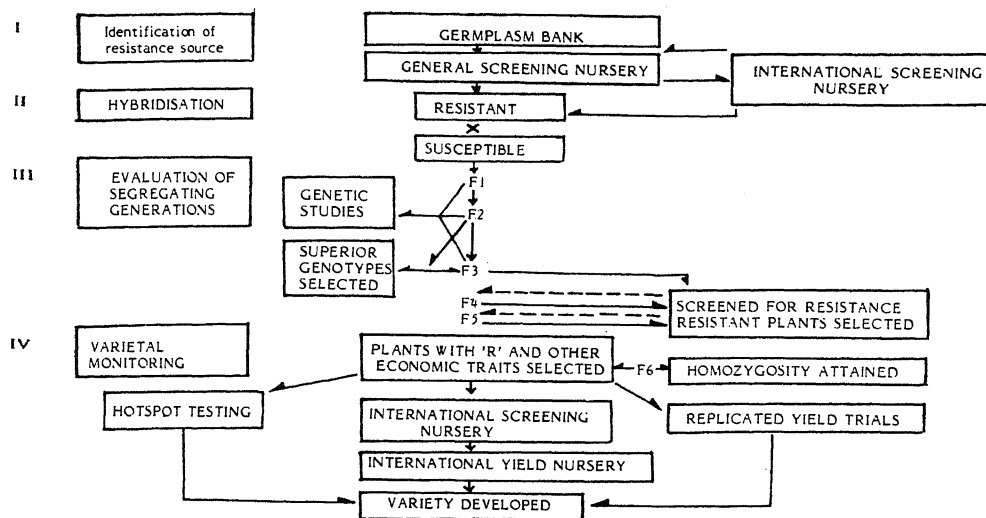


Figure 1. Development of insect resistant rice—A model.

stem borer (Devanathan 1981). Azospirillum applied to sorghum seeds and soil has been reported to impart resistance to the sorghum shootfly (Mohan *et al* 1987). This biofertilizer activates the enzyme PAL-ase and in turn synthesis of phenolics which were found to be higher in treated plots. Plants in treated plots were less infested by shootfly and this may be due to higher phenolics (Mohan *et al* 1988).

7. Application of resistance

Plant resistance is generally most appropriate and is sometimes the only practical technique for management of insect pests in crops such as cereals, pulses and vegetables. This is more so where income per hectare is low and use of insecticides is difficult. Insect resistant varieties is used in two ways: as the principal method of insect management, as an adjunct to other methods in an integrated management programme. It also avoids the release of abnormally susceptible varieties.

7.1 Plant resistance as a principal method

Insect resistant crop varieties provide an inherent control which involves no expenses nor environmental pollution problems. Even a low pest density will be controlled by a resistant variety. Hence plant resistance is now highly valued as a principal method in any pest management programme though it is not possible in all the situations. Plant resistance as a principal method is used for the management of following pests.

7.1a Rice: Insect resistant varieties have provided a highly practical approach to control the key pests such as the brown planthopper, the green leafhopper, the white backed planthopper and the gall midge. At present resistant rice varieties are cultivated not only in Tamil Nadu and other rice growing states in India but also in

many of the Asian and southeast Asian Countries. In Tamil Nadu ADT 36, Co 42, ADT 37 and PY 3 in Pondicherry are popular with the farmers and the use of insecticides to manage this pest has been remarkably reduced. MDU 3, a gall midge resistant variety is grown extensively in Periyar Vaigai Project area where the pest is a serious one. Similarly, several other varieties are grown in endemic areas particularly in Andhra Pradesh, Karnataka, Punjab and Haryana.

7.1b *Cotton*: The stem weevil was a serious problem till 1950 when the resistant variety MCU 3 was released for management of this pest. After the release of this variety the pest has been kept under check although in recent years it has assumed serious problem because of various reasons. In recent years, the whitefly *Bemisia tabaci* Gennadius has threatened the very cultivation of cotton in Andhra Pradesh, Tamil Nadu and Karnataka. LPS 141 (Kanchana), a resistant variety to the white fly was released during 1987 and the pest is under check in many areas. In addition, LK 861, Supriya are also recommended for cultivation in white fly endemic areas. In Karnataka, JK 276-4 has been released as a variety resistant to bollworms and was christened as Abhadita. In earlier years, the release of MCU 5 and MCU 9 as high yielding varieties in Tamil Nadu were also found to be resistant to the leafhopper and the pest could be easily managed without causing much damage and/or use of insecticides.

7.2 *Plant resistance as an adjunct to other methods*

The host-plants' resistance or susceptibility to pests is determined by insect-plant interactions, which are always dynamic in nature and subject to change over time and space. Hence, it is difficult to get everlasting resistance in all crops. High level of resistance, though desirable is not always essential for a crop variety to be of practical value in IPM. Therefore, varieties with moderate or even low levels of resistance can be used to good advantage for pest management. The success depends on how well other tactics are practiced such as synchronized sowing, harvesting, crop refuse destruction, appropriate cropping systems, conservation of natural enemies, selective use of safer insecticides etc.

7.2a *Insect resistance and insecticidal control*: Insect resistant varieties complement the insecticidal control. Insect population developing on resistant varieties are generally less vigorous, smaller and less fecund than those developing on susceptible varieties (Heinrichs *et al* 1984). So the susceptibility of insects developing on resistant varieties to insecticides is more. In rice, varietal resistance to stem borer and insecticidal treatments are very effective. In moderately resistant varieties, one or two applications of insecticides is found to be enough compared to 2-fold usage of insecticides on susceptible varieties. Such varieties are also found to possess a low level of resistance to other pests like GLH, Whorl maggot etc. where the resistance complements the insecticidal control. In cotton, leafhopper occurs seriously in mid and late stages of the crop in recent years. Insecticidal treatments are complementary on varieties like MCU 5 and MCU 9 while MCU 11 is highly susceptible. For management of bollworms, variety like Adhadita require less number of insecticidal treatments under irrigated conditions although the variety is resistant in rainfed ecosystems. Thus, insect resistant varieties reduce the insecticidal applications in the ecosystems.

7.2b *Insect resistance and biological control:* Plant resistance does not affect the natural enemy of insect pests. The restless behaviour on resistant varieties expose the insects to predators and parasites. Predators devour more of small sized insects on resistant plants than larger insects on susceptible hosts. In rice, a combination of varietal resistance and natural enemies gave better control of both green leafhopper and brown planthopper (Kartohardjono and Heinrichs 1984).

In cotton, the bollworm larvae hatching from eggs placed on plant apices remained longer on Frego bract cotton types and were more exposed to predators than larvae on susceptible Deltapine smooth leaf variety. Consequently, predators killed twice as many larvae on the resistant variety as on the susceptible variety. The resistant plant's morphology also facilitate predators and parasites in finding and preying upon the pest and also favour the spread of pathogens infecting the pest.

8. Genetic engineering of plant for insect resistance

In recent times, there is a growing interest in the breeding of naturally resistant plants. Here, the insecticidal proteins or genes are transferred from a resistant plant into another by way of genetic engineering. Such transformed plants are called transgenic plants, which harbour bioinsecticidal genes *in situ*. Several of the toxin genes from different strains of *Bacillus thuringiensis* have been cloned and transgenic plants produced. *Agrobacterium* mediated gene transfer into plants is also common. Recently, other plant vectors such as the Gemini tomato golden mosaic virus (TGMV) are employed to develop transgenic plants since the vectors are capable of broad spectrum infection in plants (Kunthala Jayaraman 1988). The possibility of constructing transgenic cotton plants that are resistant to the bollworms *Heliothis zea* and *H. virescens* was reported by Boulter (1989). The achievements in this emerging area of research for production of genetically engineered plants for insect control was also reviewed by Meeusen and Warren (1989). With the development of global interest and the advantages likely to accumulate, it is a promising area of research in the coming years.

9. Conclusion

Plant resistance to insects should form the foundation upon which other components of IPM should be built. Resistant varieties are effectively utilized in many countries and integrated in combination with other tactics. Growing insect resistant crop varieties is cheap and can be easily adopted by farmers. Continuous research is also essential to release newer varieties as resistant varieties are of narrow genetic base and cannot be everlasting. Cumulative resistance, derived from diverse gene pool will be more lasting and research attempts should be directed towards this line. Biotechnology, through the use of genetically engineered transgenic plants offers greatest scope and is one of the virgin areas of research with lots of potential.

Integration of insect resistance with cultural, biological and insecticidal control measures will be rewarding, in that several of the side effects including pollution in the environment will be reduced and the ecosystems maintained without deterioration.

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Biological control of weeds with insects: A dynamic phenomenon of insect-plant interaction

T SANKARAN

Biocontrol and Pest Management Consultant, 193, Gangenahalli Layout, Bangalore 560 032, India

Abstract. Extrinsic causes affecting the performance of insect enemies of weeds including climatic unsuitability, parasitism, predation and disease pathogens are discussed, giving examples of both weeds and their insect enemies selected with special relevance to India.

Keywords. Biocontrol; weeds; insect enemies.

1. Introduction

Biological control of weeds is one of the better known alternatives to chemical control and involves the deliberate use of target-specific destructive organisms to check the economic losses caused by terrestrial and aquatic weeds and other undesirable, particularly noxious, plants that occur in various parts of the world. Parasitic plants like witchweed (*Striga*), dodder (*Cuscuta*) and broomrape (*Orobanche*) are also notorious destroyers of useful vegetation. Manual and mechanical removal of weeds, cultural practices and growing of resistant strains of cultivated plants are other control methods used with varying degrees of success, depending on the severity of the weed problem and the manpower, material and monetary resources available to deal with the situation. When successful, biological control is self-sustaining, effective, economical and ecologically sound.

The biological control of the cottony cushion scale insect *Icerya purchasi* Maskell (Hem., Margarodidae) by the predacious vedalia beetle *Rodolia cardinalis* Mulsant (Col., Coccinellidae), both native to Australia, in California, USA, by the end of the 19th century led to the application of the same concept to weed control. The first weed to become a suitable target for biological control was lantana, *Lantana camara* L. (Verbenaceae), in Hawaii. This aggressive and hardy plant, originating from subtropical and tropical Central America, is now almost cosmopolitan in distribution, having been initially introduced purposefully as an ornamental shrub in most areas on account of its attractive and colourful inflorescence. However, the plant has proved toxic to livestock and even to human beings. Several insect enemies of lantana attacking various parts of the plant were found during surveys carried out at first in central Mexico during 1902 and continued from time to time later in several areas of the neotropical region for over half a century. Many of these have been introduced not only in Hawaii but in numerous other countries with partial or substantial control of the weed.

In Australia, prickly pears (*Opuntia*) were cleared over nearly 60 million acres of infested land during the 1930's by the larvae of a moth, *Cactoblastis cactorum* (Berg.) (Lep., Pyralidae) imported from Argentina. About the same time outstanding control of prickly pears was also witnessed in India but the control agent responsible for it in this country was *Dactylopius opuntiae* (Ckll.) (Hem., Dactylopiidae).

These early successes led to increasing worldwide interest in biological control of weeds. By 1980, projects covering 86 weed species and 192 biocontrol agents had been completed or were in progress in many parts of the world. More or less successful biological control has been reported with 48 different weeds in 23 locations (Julien 1982). A notable development is the utilization of native insects (and, of course, other organisms) to control indigenous as well as exotic weeds.

Sankaran (1973, 1974) reviewed the work on biological control of weeds in India. Subsequent efforts and progress and also the significant achievements made over the past one decade under the national biocontrol project of the Indian Council of Agricultural Research are summarised in a recent bulletin (Singh 1989).

A wide range of organisms from microbes to vertebrates have been used in the biological control of weeds but the present study will be restricted to insects. Further, most of the examples given here will be of special interest to entomologists and weed scientists in India who may wish to follow up the research with fresh ideas of their own.

2. Choice of phytophagous insects for weed control

Generally, alien weeds are especially amenable to biological control because of the lack of effective and host-specific natural enemies to keep them under check in the new area(s) which they have colonised. Many a wild plant species is hardly ever treated as a weed in its native distribution range, occurring at low densities, often in scattered stands, and being attacked by an assortment of insects and other natural control agents. However, when the same species invades a new area unaccompanied by its natural enemies it is able to spread and build up its population density very fast, particularly if other ecological conditions are also favourable to it. In India *Parthenium hysterophorus* L. (Compositae) is one of the best examples of such an alien weed.

In what is commonly known as classical biological control the first step is to trace the native range of the alien weed, investigate the biotic agents that regulate its population, select the most promising natural enemies, subject them to a strict protocol of host-specificity screening tests, and then to introduce the successful candidate species into the new area for field evaluation. Many of the insects attacking a potential weed in its native home may be expected to be polyphagous, which will automatically disqualify them for use in biological control in other areas in view of the risk of their attacking useful plants. In this context it should be pointed out that even in a newly colonised area an alien weed will soon be subject to attack by various polyphagous, native insects that may also be crop pests, such as *Aphis fabae* Scopoli (Aphidae) and *Ferrisia virgata* (Ckll.) (Pseudococcidae) on *P. hysterophorus* in India. It is preposterous to treat these insects as beneficial biotic agents that warrant conservation or augmentation. Host-specificity is the most essential requirement for a biocontrol agent used in weed control.

Some native insects that attack indigenous weeds and exhibit monophagy or oligophagy have been studied and used in weed control by breeding them in the laboratory and releasing them to augment the otherwise low natural populations at critical stages in the life-cycle of the weed. *Bactra verutana* Zeller (Lep., Tortricidae) has been used against *Cyperus rotundus* L. (Cyperaceae) in the USA (Frick and Chandler 1978) and periodical releases of the noctuid *Episamea pectinicornis* Hmps. are now a standard control method against *Pistia stratiotes* L. (Araceae) in Thailand (Napompeth 1982).

The use of native biotic agents may be of value against weeds for the control of which there is little or no scope for introduction of additional, more effective, biocontrol agents from other geographical areas. Although in some instances insects obtained as biocontrol agents from a climatically similar area in the native distribution range have done particularly well in the release area, this is not an indispensable condition for success because some insects have proved to be remarkably effective control agents in climatically different regions. Genetic diversity in the introduced insect population will enhance and improve the chances of success.

3. Aspects of insect-plant interaction determining host-specificity

According to Zwolfer (1973) host-specificity is largely a function of orientation stimuli. These stimuli originate from the host-plant and elicit characteristic responses from the insect acting through various types of sensory structures that have co-evolved in the course of long association and constant interaction between the insect on the one hand and the host-plant and its habitat on the other. Pieterse (1979) has cited Russian work on *Phytomyza orobanchiae* Kalt. (Dip., Agromyzidae) which demonstrated the ability of the insect to locate *Orobanche* over a radius of 3 km. With the advances made in the techniques of study on many aspects of insect-plant interaction the morphological, physiological and biochemical bases of host-specificity are being understood much better now than was possible in the first half of this century. Several valuable contributions to our knowledge of this subject have emanated from the Entomology Research Institute, Madras (Ananthakrishnan 1977, 1986).

Some groups of insects contain genera and species that have very specialised host-plant associations and correlated adaptive mechanisms. However, very little is known of all the factors responsible for such insect-plant affinities. For example, why are many species of the weevil genus *Smicronyx* restricted to parasitic plants such as *Cuscuta* (Convolvulaceae) and *Striga* (Scrophulariaceae)? Species of another weevil genus, *Nanophyes*, that produce and breed in the fruit-galls of their host-plant also exhibit a high degree of specificity as evidenced by a complex of species that occur on various *Ludwigia* spp. (Onagraceae) in India (Sankaran and Rao 1972). The biology of *Nanophyes* sp. nr. *nigritulus* Boh. studied by Sankaran and Krishna (1967) revealed a remarkable synchronisation between the phenology of its host-plant *Ludwigia adscendens* (L.) Hara (= *Jussiaea repens* L.) and the ontogeny of the insect.

Insects selected for use in biological control of weeds may be restricted to their host-plants by their oviposition habit, nutritional needs as well as larval developmental requirements, and pupation habit.

Morphological peculiarities or physiological or biochemical cues of the host-plant (actual oviposition site) may attract the mature female to lay eggs and it is sometimes possible to induce a female to oviposit on unnatural plants simulating natural conditions but larval development will not be completed on or within such plants. In screening tests at CIBC, Bangalore, the weevil *Neochetina eichhorniae* Warner, which has now been so successfully used in the control of water hyacinth *Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae) in some parts of India and in many other countries, oviposited on water chestnut, *Trapa bispinosa* Roxb.

(Trapaceae) but the larva failed to complete its development on it although it fed slightly on the leaf for 10 days.

However, habitual oviposition by an insect on or within a plant species is not by itself an exclusive proof of host-specificity. *Gesonula punctifrons* (Stal.) and *Paulinia acuminata* (De Geer), both grasshoppers, have been studied for their host-specificity and evaluated as biocontrol agents. *G. punctifrons* makes tunnels in the soft tissue of the petiole of water hyacinth and lays eggs inside but the nymphs and adults feed not only on water hyacinth but also on *Arum* (Araceae), *Colocasia* (Araceae) and *Monochoria* (Pontederiaceae) and some unrelated economic plants (Sankaran *et al* 1966). In laboratory tests it was also found to make oviposition tunnels in the stem of *Canna* (Cannaceae). It is an Oriental species that has become adapted to the alien (neotropical) water hyacinth. The neotropical grasshopper *Cornops longicorne* (Bruner) has oviposition habit similar to that of *G. punctifrons*, a good example of convergent evolution. *P. acuminata* is another neotropical grasshopper that oviposits on the submerged undersides of the leaves of *Salvinia molesta* D S Mitchell (Salviniaceae) and the eggs do not develop normally and hatch if continuously exposed to the atmosphere. This habit confines it to an aquatic environment, where it may also feed on a few other aquatic plants.

Dietary needs of even host-specific insects may be satisfied not only by the food-plants preferred by them in nature but also by one or more other species of plants. This faculty has helped in the development of artificial diets to breed several species of insects in the laboratory. In screening tests carried out with some phytophagous insects that have eventually been introduced into new areas for weed control the larvae or adults were found to feed on a few related or unrelated plants. However, the adults failed to reproduce after feeding on such plants. In very rare instances the candidate insect fed and also reproduced on the test plant, for example the lantana bug *Teleonemia scrupulosa* Stal. (Hem., Tingidae) on *Tectona grandis* L. (Verbenaceae). The lantana bug has, nevertheless, not become a pest of teak although it has co-existed with teak in India since at least 1951. Davies and Greathead (1967) reported that *T. scrupulosa* attacked *Sesamum indicum* L. (Pedaliaceae) in Uganda but the bug is not known to be a pest of sesame in India and Mexico (its original home) where this crop is widely cultivated. Three other lantana insects, *Leptobyrsa decora* Drake (Tingidae), *Octotoma scabripennis* (Guerin) and *Uroplata girardi* Pic. (both Col., Hispidae) are also capable of feeding on sesame (Harley 1969; Harley and Kassulke 1971).

An aspect of host-specificity that merits much closer investigation is the occurrence of more than one congeneric species feeding on different parts of the same plant. The larvae of the noctuid *Eulocastra argentisparsa* Hmps. feed on the ripening seeds in the fruit-pods of *Striga* spp. while those of the closely related *Eulocastra undulata* Snellen feed on the leaves. The larvae of these two species differ in their colouration but the moths reared from them were at first identified by the Commonwealth Institute of Entomology as *E. argentisparsa* (Sankaran 1973). The water hyacinth weevils *N. eichhorniae* and *Neochetina bruchi* Hustache are both able to thrive together because although the larvae have similar feeding habits the adults show different feeding preferences and temperature tolerances and the oviposition behaviour of the females of the two species also differs (Singh 1989).

The mature larvae of some insects used against aquatic weeds seek specific niches as pupation sites that are characteristic of their host plants. *Agasicles hygrophila* Selman and Vogt (Col., Chrysomelidae) successfully introduced into Australia and

the USA from Argentina to control the alligator weed *Alternanthera philoxeroides* (Mart.) Grisebach (Amaranthaceae), though an external feeder in its larval stages (and also as adult), chews its way as mature larva into one of the apical internodes above the water level and pupates inside. The newly formed adult feeds on the internal side of the stem wall for a short while and then emerges. Full-grown larvae of the two *Neochetina* spp. come out of their feeding tunnels and pupate in submerged cocoons covered over by a ball of root hairs of water hyacinth. Similarly *Cyrtobagous salviniae* Calder and Sands, another weevil that has controlled *Salvinia molesta* in India, Australia etc. pupates within a submerged cocoon in close contact with the plant tissue. Ability to pupate in a non-aquatic medium may render a species suspect and necessitate additional host-specificity studies. The tuber weevil *Bagous affinis* Hustache, introduced recently into the USA from India to control *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) feeds on hydrilla stems but the larvae later complete their feeding and pupate within the tuber although pupation may also take place in soil with adequate moisture (Buckingham 1988).

4. Insect damage potential and its impact on weeds

Insects that damage different parts of the same weed may kill a weed or contribute to an overall reduction in its growth, vigour and reproductive potential. Therefore, if one species fails to control a weed adequately others may be used to enhance the level of control. Synergistic action by two species may also inflict more damage on the weed than that possible by either species working alone, as with *N. eichhorniae* and the water hyacinth mite *Orthogalumna terebrantis* Wallwork (Del Fosse 1978). Species that severely damage the root system or the main stem may kill the plant within a short time while defoliators and flower feeders are less drastic in their effect. Both annual and perennial weeds have been successfully controlled biologically by employing insects. However the intrinsic damage potential of every species may not be fully realised in all situations. Environmental constraints may operate against them. Most of the phytophagous insects themselves are subject to parasitism, predation and pathogenic diseases, and as a result many species occur at low populations, particularly at the most vulnerable stages in the life-cycle of the weed. With annual weeds that only propagate by seed, fruit- and seed-feeding insects are likely to be more effective control agents than they are with weeds that propagate vegetatively as well as by seed.

Larvae of the noctuid *Eulocastra argentisparsa* cause complete loss of seed in individual *Striga* plants but their natural populations are always very low. A microsporidian disease is known to infect them. Moreover *Striga* is an annual weed and its seed-maturing phase is short. *Eulocastra* is unable to build up its population early enough to reduce the total seed output in an infested area significantly. It has been suggested that the insect may be mass-bred on an artificial diet in the laboratory for use in biological control after the necessary screening tests.

A gall-forming weevil, *Smicronyx albovariegatus* Fst. attacks *Striga* spp. in India but most of the galls are produced on the root, stem and branches and yet do not impair the vigour or reproductive capacity of the host-plant. The host-plant is able to withstand, if not resist, the weevil attack. Some other *Smicronyx* occurring in Africa produce fruit galls and thereby arrest seed production. However, parasitism limits their value as control agents.

Larvae of the tortricids *Bactra* (*Nannobactra*) *minima minima* Meyr. and *Bactra* (*Chiloides*) *venosana* Zeller bore into the stem and occasionally into the rhizome of *Cyperus rotundus* L. in India but, since even a partially damaged rhizome can produce a fresh shoot, the insect is not able to kill the plant. The new shoot may also be infested by the borer. Such plant regeneration and insect counter-attack have the final effect of dwarfing the plant by keeping it under constant stress, which gives cultivated plants a competitive advantage over the weed in crop fields. Here again various parasites reduce the effect of these borers (Sankaran and Rao 1972).

The gall-fly *Procecidochares utilis* is widely established on *Eupatorium adenophorum* in many parts of India but its control action is weakened by native parasites.

Harris (1973) has discussed many of the diverse interspecific and intraspecific mechanisms that contribute to low or high vulnerability of weeds to damage by biocontrol agents, with particular reference to photosynthetic activity and carbohydrate reserves in the plant, and to flowering, plant density and competition.

5. Success or failure of insect enemies in biological control of weeds

Many species of insects introduced from one part of the world into another for weed control that have failed to become established have hardly received any further attention although there is much to learn from the negative results of biological control attempts. Several others that are established but do not exert significant levels of control on the target weed in some area(s) have been tried elsewhere with better success. A number of species have performed remarkably well in different areas wherever they have been tried. The latter two categories are preferred for use in newer areas without or after additional testing. However, taking insects and all other biocontrol agents into account, 60–75% of the introductions for weed control are considered to be ineffective (Julien 1982).

The causes for failure of a species may be extrinsic, i.e. they are not directly related to insect-plant relationship *per se* but attributable to unfavourable factors of the environment such as climatic rigours, interference from natural enemies, man-made disturbances etc. The gall-fly *P. utilis* well established on *E. adenophorum* in the Nilgiris is handicapped by the high annual rainfall in the area, apart from being parasitised by native chalcidoids.

The critical importance of infraspecific biosystematics to biological control of weeds has been amply demonstrated in recent years. Investigations in Australia have revealed that lantana naturalised there for over a century occurs in some 21 strains or varieties but *T. scrupulosa* has definite preferences for particular taxa, which reduces its control value in areas where other taxa predominate. This is thought to be due to the limited gene pool of the original stock of the insect imported in 1935. Enrichment of the genetic foundation of the local population by incorporating fresh stocks of the bug from various parts of south America resulted in better control of the resistant varieties of lantana (Harley 1973).

Pareuchaetes pseudoinsulata Rego Barros (Lep., Arctiidae), previously known as *Ammalo insulata* Walker, introduced from Trinidad into several countries to control *Chromolaena odorata* (L.) (Compositae), presents a puzzling picture of contrasting performance. It failed to get permanently established in Ghana, Malaysia and Nigeria. In Ghana, the larvae were predated upon by insects, lizards and spiders. In

Sabah (Malaysia) it did not persist for long. The causes for its failure there and in Nigeria are unknown. Large-scale field releases of eggs, larvae and adults in the plantations of Kodagu (Coorg) in Karnataka did not result in success. Native predators, mainly ants, were largely responsible for its failure. The ants were attracted to the weed because of the presence of honey-dew-producing aphids and mealybugs. A granulosis virus of the larva is also believed to have prevented its establishment. In renewed efforts, a strain of the same insect was obtained from Sri Lanka, where it had been introduced from Trinidad in 1973 and become naturalised, and was released in a rubber plantation in Kerala and it became established. Defoliation of the weed has occurred over a small area (Singh 1989). In marked contrast, the same strain introduced from India in 1985 was soon established on the island of Guam (Pacific Ocean) by 1986 and has destroyed the weed over 10,000 acres (CIBC Report, April–December 1986).

The level of biological control resulting from interaction between an agent and the weed can be raised by environmental stresses acting on the plant. Harris (1981) has suggested a 5-fold strategy to increase the stress on the weed: (i) decreasing the mortality factors affecting the control agent, (ii) increasing the number of agents attacking the weed, (iii) increasing the fecundity of the agent, (iv) altering cultural practices to stress the weed while still favouring the agent and (v) selection of agents that inflict a high stress load. These include even unconventional measures such as limited use of insecticides to eliminate predators that interfere with biological control by phytophagous agents and increasing the nutritive value of the weed by correcting mineral and nitrogen deficiencies in the habitat by judicious application of chemical inputs. These measures will make biological control an important component of weed management.

6. Conclusions

Biological control of weeds by insects, whether native species or introduced ones, involves a complex of interacting morphological, biochemical, phenological and other factors in both host-plant and control agent. Host-specificity is the most essential requirement for an insect to be used as a weed control agent. While an insect initially locates its preferred host-plant by responding to various orientation stimuli, eventually it selects and remains on the plant for feeding and breeding by reacting more intimately to one or more of the various factors mentioned above. Oviposition habit and nutritional suitability of the plant for growth and reproduction are important criteria in determining insect-plant association. Insects attacking water weeds have characteristic biological traits that tie them to an aquatic habitat. Some taxa of weeds are resistant to or tolerant of attack by certain insects but may be susceptible to damage by different agents from other areas. More than one species of insect occurring on the same weed at the same time but exhibiting complementary and not competitive feeding and oviposition habits may have a synergistic effect in suppressing the weed. Apart from climate, biotic factors such as parasites, predators and pathogenic organisms, as well as biochemical and phenological changes in host-plant vulnerability are among the major causes that affect the control value of insect enemies of weeds. Measures to eliminate or minimise their adverse impact are known and can be adopted to improve biological control.

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Impact of host food plant on parasitization behaviour in a larval parasitoid of *Heliothis armigera* (Hubner)

T V SATHE

Department of Zoology, Shivaji University, Kolhapur 416 004, India

Abstract. The sequence of host seeking and stimuli involved in them for the larval parasitoid was *Eriborus argentipilosus* and its host *Heliothis armigera* are discussed through a series of experimental observations. In such a sequence of behaviour the step-by-step release of the different responses tending to contribute to the efficiency of host finding are explained.

Keywords. Parasitization behaviour; larval parasitoid; *Heliothis armigera*; Parasitoid-host-plant system; insect-plant relationship.

1. Introduction

As parasitoids are important for the regulation of host populations and their use in the biological control of insect pests, there is a strong and persistent interest in the population dynamics of parasitoid-host-plant systems. Various stimuli aid entomophagous parasitoids in finding and recognizing hosts. In the host selection of parasitic hymenoptera, usually visual and/or chemotactile stimuli (Vinson and Lewis 1965; Arthur 1966; Corbet 1971) are involved.

The role of chemicals in insect-plant relationship has received much attention, the importance of other cues and sensory modalities has gone relatively unexplored (Herrebout 1969). Very few workers have published the data concerning the chemical stimuli that elicit parasitization behaviour. Noteworthy among them are Dethier (1947), Arthur (1969), Arthur *et al* (1972), etc. The chemical stimuli in such behaviour have been identified (Vinson *et al* 1975). Vinson (1976) outlined specific mechanisms whereby parasitoids may be able to utilize oligophagous hosts on only a part of their food plant range. However, these chemicals have usually not been behaviourally assayed as a part of the host finding sequence, notwithstanding that they are assumed to be not merely attractive but also to play a part in controlling different steps of the behaviour.

The specificity of behavioural response exemplified by oligophagous insect species for odours typifying their host plants, suggests some specialized tuning of their olfactory receptors located in the antennae. The evidence has been presented indicating that the oligophage responds to a specific blend of green leaf volatiles, released by potato (Richerson and Borden 1972). Jones *et al* (1973) have reviewed earlier work on host odours. The kairomones are transspecific chemical messengers, the adaptive benefits of which favour the recipients rather than the emitters (Brown *et al* 1970). Literature on the role of kairomones in host finding of entomophagous parasitoids is accumulating.

The visual stimuli are the most important factors in host selection and the parasitoid attacks, if the host is moving and is red or black (Richerson and Deloach 1972). Some species can recognise a suitable host by piercing it with the ovipositor (Salt 1935; Jackson 1928; Weseloh 1974; Wylie 1965). While, some species detect

their hosts by perception of sound or vibrations produced by host larvae (Deleon 1935). The host finding is also related with thermal (temperature) or infrared radiation stimulus (Richerson and Borden 1972).

The subcortical and wood boring insects find and oviposit into or on hosts through plant tissues. However the mechanisms are little understood (Richerson and Borden 1972).

Generally, the female parasitoids are attracted by the food plant leaf of their host and are stimulated by it to start searching; further stimulation from new damage by host larvae releases excitement behaviour and continued intensive searching (Sato 1979). In the close vicinity of a host, orientation of antennae occurs and finally stabbing. In such a sequence of behaviours, the step by step release of the different response may contribute greatly to the efficiency with which hosts are found. The present study analyses both, the sequence of host seeking and the stimuli involved in them for the parasitic wasps, *Eriborus argenteopilosus* (Cameron) and its host, *Heliothis armigera*.

2. Materials and methods

The laboratory cultures of *E. argenteopilosus* was used. The experiments were conducted in glass cages and test tubes (19×2.5 cm) at room temperature ($25 \pm 1^\circ\text{C}$ and $60 \pm 6\%$ RH). In each experiment 20 mated female parasitoids were exposed. Because of the diverse nature of the experiments, methods and results of each of the foregoing experiments are presented separately wherever appropriate. The adult parasitoids were fed with 50% honey and host larvae with respective food plant parts. The main food plants of the *Heliothis* was grown out of doors. Other food plants were tested as indicated in the text, either cultivated in the laboratory or collected from the field during the experiments. The leaf pieces measuring 1.5×1.5 cm were used in the experiments.

3. Results

3.1 Behaviour of female on food plant: sequence of host seeking behaviour

The young pigeon pea plant (1-month old) was kept in a glass cage and observations on the seeking behaviours of 20 mated females were made.

Firstly, she came in contact with the leaf, further performed walking movements over the leaf and tapped the leaf surface 2–3 times with antennae. Then raised the wings and performed movement of third pair of legs. When she came across the food plant leaves damaged by host larvae, has performed intensive searching and stabbing intention movements. The presence of a 2nd instar host larva immediately brought about oviposition. When landed on a healthy leaf she searched for about 22" and then flew away.

3.2 Food plant factors eliciting searching behaviour

3.2a Responses to different food plant leaves (Expt. 1): For the experiment chick pea, pigeon pea, lady's finger, cabbage, cauliflower and tomato were used as host

food plants. Pieces of respective food plant leaf (about 1.5×1.5 cm except for chick pea 1×0.5 cm) were kept in each test tube with a female parasitoid and observations were noted. With pigeon pea leaves maximum (100%) females have performed searching movements (mean search: $34.75 \pm 6.77''$) while, with cabbage leaf piece minimum searching (45%) was recorded (mean search: $10.44 \pm 2.72''$). None of the parasitoids have performed searching movements on chick pea leaf (table 1).

3.2b *Responses to females to food plant parts (Expt. 2):* To find out the favourite searching site of female parasitoids, pigeon pea leaf, flower bud and pod were cut into same size (1.5×1.5 cm) and exposed to them. The results (table 2) indicate that 100% females have searched on leaf piece while 75% of them have searched on flower bud and 60% on pod. As the optimum searching was noted on leaf pieces (mean search: $34.75''$) hence, used in further experiments to observe the behavioural responses.

3.2c *Responses to cellophane wrapped leaves (Expt. 3):* Pieces of pigeon pea leaf (1.5×1.5 cm) were wrapped with cellophane and exposed to female parasitoids. The parasitoids have not responded to them. The wrapped leaf was neither attractive nor repulsive. It seems that females were unable to locate a food plant leaf by visual stimuli.

3.2d *Responses to collodionized leaves (Expt. 4):* With the exposure of collodionized leaves, 25% female parasitoids have performed searching movements. When these leaves were washed with ether and their further exposure resulted in searching in 80% of the parasitoids (table 3). These parasitoids have searched for a mean period of $10 \pm 2.19''$ and $21.65 \pm 2.79''$. After removing the ether the females have shown orientation of antennae, vigorous movements of third pair of legs,

Table 1. Responses of female, *E. argenteopilosus* to different food plant leaves of *H. armigera*.

Food plant	n	Females searched (%)	Mean search \pm SE
Pigeon pea	20	100	$34.75 \pm 6.77''$
Chick pea	20	0	—
Tomato	20	80	$25.15 \pm 4.77''$
Lady's finger	20	80	$17.85 \pm 4.67''$
Cauliflower	20	70	$12.6 \pm 4.66''$
Cabbage	20	45	$10.44 \pm 2.72''$

Table 2. Responses of female, *E. argenteopilosus* to host food plant parts.

<i>C. cajan</i>	n	Females searched (%)	Females performed stabbing intention movements (%)	Mean search \pm SE
Leaf	20	100	—	$34.75 \pm 6.77''$
Flower bud	20	75	—	$21.4 \pm 3.53''$
Pod	20	60	—	$14.08 \pm 3.45''$

Table 3. Responses of female, *E. argenteopilosus* to collodionized pigeon pea leaf.

Leaf	n	Females searched (%)	Mean search \pm SE
Clean (control)	20	100	$34.75 \pm 6.77''$
Collodionized	20	25	$10.0 \pm 2.19''$
Collodion ether removed	20	80	$21.65 \pm 2.79''$

Table 4. Responses of female, *E. argenteopilosus* to filter treated variously with ether extracts of pigeon pea leaf.

	n	Females searched (%)	Females performed stabbing intention movements (%)	Mean search \pm SE
Paper with extract of intact healthy leaf	20	100	85	$24.55 \pm 4.7''$
Paper with ether alone	20	0	0	—

followed by flying movements toward the leaf. Further, they performed vigorous searching movements, but did not exhibit stabbing intention movements.

3.2e *Responses to leaf extracts on filter paper (Expt. 5):* The results (table 4) showed that all females (100%) have performed searching movements (mean search: $24.55 \pm 4.7''$) by orientation of antennae and vibrating the wings. Eighty five per cent of them have shown excited behaviour and followed by stabbing intention movements. Females did not show response to filter paper treated with ether alone. The factors responsible here for bringing the intensive searching and stabbing intention movements were chemical.

3.3 Damaged leaf factors eliciting responses

3.3a *Responses to leaves damaged by H. armigera larvae (Expt. 6):* With the leaves of pigeon pea, chick pea, lady's finger, cauliflower, cabbage, tomato damaged by host, females have performed absolute searching (100%) and stabbing intention (100%) movements on pigeon pea leaf (mean search: $50.5 \pm 12.02''$). The minimum searching and stabbing intention movements were recorded with tomato leaves (mean search: $20.2 \pm 6.71''$). Whereas, female parasitoids did not show searching and stabbing intention movements on chick pea leaves (table 5).

3.3b *Responses to damaged leaf extracts on filter paper (Expt. 7):* All females have performed intensive searching movements by orienting and drumming movement of antennae, vigorous leg movements, vibrating the wings (mean search: $41.75 \pm 3.76''$). Eighty per cent have performed stabbing intention movements by performing up-and-down movements of abdomen, vigorous vibration of wings, etc. With the exposure of extract of an intact leaf cut into the filter paper size, only 70% females

Table 5. Responses of female, *E. argenteopilosus* to leaves damaged by larvae, *H. armigera*.

	n	Females searched (%)	Females performed stabbing intention movements (%)	Mean search ± SE
Pigeon pea	20	100	100	50.5 ± 12.02"
Chick pea	20	0	—	—
Lady's finger	20	80	45	33.65 ± 7.98"
Cauliflower	20	85	30	42.35 ± 4.63"
Cabbage	20	60	25	32.1 ± 4.31"
Tomato	20	40	15	20.2 ± 6.71"

Table 6. Responses of female parasitoids to damaged leaf extracts on filter paper.

	n	Females searched (%)	Females shown stabbing intention movements (%)	Mean search ± SE
Filter paper + ether extracts of damaged leaf	20	100	80	41.75 ± 3.76"
Filter paper + ether alone	20	—	—	—
Extract of cut leaf	20	70	45	25.2 ± 7.39"
Cut leaf	20	100	—	18.4 ± 4.20"

have performed searching movements (mean search: $25.2 \pm 7.39''$) and 45% of females have shown stabbing intention movements (table 6). The chemical stimuli that released from damaged leaf extract were responsible in bringing the searching and stabbing intention movements in female parasitoids.

3.3c Responses to objects treated with saliva of host larvae (Expt. 8): The females have shown 100% searching and 85% of them exhibited stabbing intention movements on the punched region of pigeon pea leaf (table 7). With saliva treated along with leaf edges, all parasitoids have performed searching movements (mean search: $28.6 \pm 7.5''$) and 75% of them have shown stabbing intention movements. When females came across with leaf piece whose surface was treated with host larval saliva, 85% of them have shown searching movements (mean search: $25.5 \pm 4.04''$) and 60% have performed stabbing intention movements. The filter paper which was treated in the above method was relatively less effective to saliva treated on leaves of pigeon pea (table 7). The factors that brought the exhibition of searching and stabbing intention movements were again olfactory.

3.3d Responses to larval faeces (Expt. 9): With the fresh wet faeces all females have performed intensive searching movements (mean search: $52 \pm 10.39''$) and 95% of them have shown stabbing intention movements. The dry faeces were

Table 7. Responses of female, *E. argenteopilosus* to objects treated with saliva of host larvae.

Treatment/saliva applied	n	Females searched (%)	Females shown stabbing intention movements (%)	Mean search \pm SE
Pigeon pea leaf				
Edge	20	100	75	28.6 \pm 7.5"
Around hole	20	100	85	33.85 \pm 8.45"
Surface	20	100	60	25.5 \pm 4.04"
Leaf without saliva	20	100	—	35.74 \pm 6.77"
Filter paper				
Edge	20	70	45	23.2 \pm 4.06"
Around hole	20	90	55	23.3 \pm 7.18"
Surface	20	25	25	21.5 \pm 5.59"
Filter paper without saliva	20	—	—	—

Table 8. Responses of female, *E. argenteopilosus* to faeces.

	n	Females searched (%)	Females performed stabbing intention movements (%)	Mean search \pm SE
Fresh, wet faeces	20	100	95	52.0 \pm 10.39"
Dry faeces	20	80	55	18.7 \pm 4.06"

relatively less effective, because 80% of females have performed searching movements (mean search: 18.7 \pm 4.06") and 55% of them have performed stabbing intention movements. The olfactory stimuli that emanated from faeces of host larva were responsible for intensive searching and stabbing intention movements of female parasitoids (table 8).

3.3e *Responses to filter paper treated with pigeon pea leaf juice and saliva* (Expt. 10): With pigeon pea leaf juice and saliva, 100% females have performed intensive searching movements (mean search: 40.35 \pm 5.84") and 75% of them have shown stabbing intention movements. Similarly, with the control filter paper, all female parasitoids have performed intensive searching movements (mean 23.35 \pm 3.22") but, only 15% of them have shown stabbing intention movements (table 9).

3.4 Host larval factors eliciting attacking behaviour

3.4a *Responses to host larvae* (Expt. 11): The close vicinity of a host larva brought about intensive searching movements and excitement in female parasitoids. By contacting the host larva female thrusts ovipositor in host's body by bending

Table 9. Responses of female, *E. argenteopilosus* to filter paper treated with pigeon pea, leaf juice and saliva of host larva.

	n	Females performed searching movements (%)	Females performed stabbing intention movements (%)	Mean search \pm SE
Leaf juice	20	100	15	23.35 \pm 3.22"
Leaf juice + saliva	20	100	75	40.35 \pm 5.84"

the abdomen. The mean searching period among female was of 45". Their mean delay to a first oriented stabbing was 10.4 ± 2.4 ".

Further, the host larvae were exposed to female parasitoids directly without any food plant leaves. The female was able to recognise the larvae only when they were in 2–4 mm distance. Female performed orientation of antennae and up-and-down movements of abdomen. Further, she moved closer to the host larva, grasped it and inserted her ovipositor into the abdominal segments of host's body. The average time required for first stabbing was 18.2 ± 3.8 ". The factors responsible were both visual and olfactory in nature.

3.4b Responses to host larva wrapped in cellophane (Expt. 12): The female parasitoids have not performed any searching or exciting movements with host larva wrapped in cellophane. The results indicate that visual stimuli alone were not sufficient to bring about parasitization behaviour in the parasitoids.

3.4c Responses to host larvae in the dark (Expt. 13): The females have identified and oviposited in the host larva as they did in the presence of light. The stabbing occurred in 22.8 ± 2.2 ". The factors responsible here were olfactory.

3.4d Responses to leaves contaminated with host's odour (Expt. 14): All females have performed vigorous searching and stabbing intention movements on the leaf surface. The mean searching period was 52 ± 3.4 ". The females have performed vigorous antennal movements, leg movements and up-and-down abdominal movement, while doing so they brought their ovipositor in contact with leaf surface. The factors responsible were olfactory.

3.4e Responses to filter paper treated with extracts of host larvae (Expt. 15): The filter paper treated with ether extract of host larva was more effective than the ether washed larva. With ether extract, all females have performed searching and 75% of them have shown stabbing intention movements (mean search: 24.1 ± 2.38 "). Whereas, only 60% of female have performed stabbing intention movements (mean search: 13.55 ± 2.81 ") on larva washed with ether (table 10). The above results indicate that odours were mainly responsible for eliciting searching and stabbing intention movements in female parasitoids.

4. Discussion

The main objective of a female parasitoid should be to locate and oviposit in or on

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Filter paper without saliva	20	—	—	—

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Further, the host larvae were exposed to female parasitoids directly without any food plant leaves. The female was able to recognise the larvae only when they were in 2–4 mm distance. Female performed orientation of antennae and up-and-down movements of abdomen. Further, she moved closer to the host larva, grasped it and inserted her ovipositor into the abdominal segments of host's body. The average time required for first stabbing was 18.2 ± 3.8 ". The factors responsible were both visual and olfactory in nature.

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4. Discussion

The main objective of a female parasitoid should be to locate and oviposit in or on

Table 10. Responses of female, *E. argenteopilosus* to filter paper treated with extracts of host larvae.

	n	Females performed searching movements (%)	Females performed stabbing intention movements (%)	Time to first stab	Mean search \pm SE
Intact larva	20	100	100	10.4 \pm 2.4	13.55 \pm 2.81"
Larva washed with ether	20	100	60	18.24 \pm 1.4	16.83 \pm 1.72"
Filter paper + ether extract	20	100	75	16.46 \pm 2.1	24.1 \pm 2.38"
Filter paper ether	20	—	—	—	—

to its host. Unless emergence occurs alongside a suitable host, the parasitoids respond to a series of environmental cues which lead the parasitoid towards appropriate habitat, host locality (food plant) and host and stimulate to oviposit (Vinson 1977).

The experimental proof of habitat selection was provided by Laing (1937) in the braconid, *Alysia manducator* Panzer. Attraction of ovipositing females to the food plant of phytophagous hosts has been shown for the ichneumonid, *Pimpla ruficollis* (a parasitoid of Lepidoptera larvae) (Thorpe and Caudle 1938) and among the Diptera for the tachinid, *Drino bohémica* Mesn parasitoid of sawfly larvae (Monteith 1955, 1964), and *Eucarcelia rutilla* Vill, a parasitoid of Lepidopteran larvae (Herrebout and Van den Veer 1969). Stray (1964) described the role of aphid's host plant for those parasitoids in the family Aphidiidae. Mackauer (1965) suggested that aphid parasitoids may respond to signs of aphid damage and presumably find the hosts habitat by visual means.

The present observations demonstrate that *E. argenteopilosus* females were attracted by the food plant leaf of their hosts and were stimulated to start searching, further stimulation from new damage by host larvae released excitement behaviour and continued intensive searching. In the close vicinity of a host, orientation of antennae occurs and finally stabbing. In such a sequence of behaviours, the step-by-step release of the different responses might contribute greatly to the efficiency with which hosts were found.

The experimental analysis revealed that the initial attraction to the food plant leaf was made by olfactory stimuli emitted from it. Once on a leaf, the odour of the plant then elicits searching. Intensive searching, however, was not elicited without the nearby presence of leaf damage by host larvae. The factors responsible were olfactory rather than visual. The odour was due to the combination of leaf juices and host saliva, perhaps chemical breakdown product of the two. That odour elicited excitement and stimulated stabbing intention movements even without the presence of host larvae. A host larva itself always attracted a nearby female wasp but only over a few mm. The factor was again not visual but olfactory.

The females of parasitoid discussed were also stimulated by host larval saliva and faeces. The variety of effective stimuli have not confused females, but actually improved their chances of finding a host by eliciting intensive searching, the most essential for which was odour from damaged leaves. Such damage was continually

renewed as long as host larvae were feeding, so the response to the fresh damage had a specific locating function.

The odour of a food plant leaf elicited searching, but the searching lasted for about 10" only unless other stimuli elicited later components in the sequence. Thus, in the presence of a host larva, females came across a damaged part of a leaf and switched to intensive searching, which would then lead to an encounter with the host larva. When host larvae were not present, a female will therefore, soon takes off without wasting time on further unprofitable searching. The above observations indicate that in *E. argenteopilosus* olfactory stimuli were responsible in attracting female parasitoids towards host food plant/host larvae.

The chick pea plants secrete oxalic acid over their surface—probably the odour of this acid might be responsible for attracting *C. chlorideae* towards this plant. It was also noted that per cent parasitism of *H. armigera* was nil on chick pea by *E. argenteopilosus* while *C. chlorideae* showed high per cent parasitism on the same. The odours emitting from the food plants might be responsible in attracting/repelling female parasitoids and further to increase/decrease the per cent parasitism in the field conditions.

There is a need to identify and synthesize the chemicals involved in the process of parasitization behaviour. These chemicals would be useful in pest management programmes for attracting parasitoids into crops and for stimulating and prolonging searching behaviour.

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Bioenergetics in insect-plant interactions

J MUTHUKRISHNAN

School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Abstract. Homeostatic mechanisms involving the regulation of the rates and efficiency of food consumption and utilization are reviewed. Adjustment in the rates and efficiencies of consumption and utilization of food during switching on from one host plant to another as well as the compensatory tactics among the consumer species are discussed.

Keywords. Plant chemical composition; insect bioenergetics.

1. Introduction

The origin of phytophagous insects from their detritivorous ancestors and the emergence of terrestrial plants from aquatic habitat and their establishment on land took place simultaneously (Southwood 1972). The phytophagous insects found a better source of nutrients in terrestrial plants than in the nutritionally poor detritus, on which their ancestors were dependent. Consequently, the plants were subjected to a heavy feeding pressure not only by the insects but also by vertebrates. Since then the chemical war between the coevolving groups of plants and insects resulted in a series of morphological, physiological and ecological adaptations in them (Feeny 1976). Many plants have modified their metabolic pathways and evolved a variety of toxic secondary chemicals in defense against herbivores (Schoonhoven 1981). On the other hand, in addition to improving their chemosensory system to enhance host finding and host assessment efficiency, the insects evolved detoxification mechanisms (mixed function oxidases) to counteract the toxic plant chemicals (Brattsten 1979) and homeostatic mechanisms involving regulation of the rates and efficiencies of consumption and utilization of food, to meet their qualitative and quantitative nutritional requirements (Scriber and Slansky 1981).

The spectrum of host plants ingested by insects consists of 10 or more families for generalists (=polyphagous), 2–10 families for intermediates (=oligophagous) and only one family for specialists (=monophagous) (Scriber 1983). Irrespective of the width of the spectrum of host plants, host finding, assessment and acceptance mostly depend on nutrient composition especially protein, water and allelochemicals. When adequate concentrations of the nutrients are not available in the host plant, the insects switch over from one host to other or from one part of the host to other of the same host such as leaf to flower or seed. The switch over is generally followed by adjustments in the rates and efficiencies of consumption and utilization of food. Occasionally, availability of the preferred host plant may be restricted calling for compensatory tactics among the consumer species (Muthukrishnan and Delvi 1974; Muthukrishnan *et al* 1978). The present article highlights the influence of insect-host plant interactions on bioenergetics.

1.1 Bioenergetics

It deals with quantitative aspects of food consumption (C), egestion (F), excretion (U), assimilation (A), metabolism (R) and production (P = conversion) by cells, organisms or populations. The IBP formula of Petruszewicz and MacFayden (1970) usually represented as

$$C = F + U + R + P, \quad (1)$$

is followed in bioenergetics studies. Although energy is not a primary requirement for phytophagous insects, acquisition of sufficient food energy is determined by the various phagostimulants (e.g. protein, water, sugar etc.) and feeding deterrents (e.g. secondary plant substances) present in the food, which evoke positive or negative feeding responses in the consumer. Gravimetric method (Waldbauer 1968) is followed for the quantitative estimation of C , F and P in terms of dry weight, which is converted into energy terms considering the energy equivalents of appropriate samples. Assimilation (A) is calculated as the difference between C and F

$$A = C - F, \quad (2)$$

and metabolism (R) as the difference between A and P .

$$R = A - P. \quad (3)$$

Efficiencies of assimilation (Ae) and gross (Pe_1) or net (Pe_2) production are calculated by relating A to C and P to C or A respectively and expressed in percentage.

$$Ae(\%) = \frac{A}{C} \times 100. \quad (4)$$

$$Pe_1(\%) = \frac{P}{C} \times 100. \quad (5)$$

$$Pe_2(\%) = \frac{P}{A} \times 100. \quad (6)$$

Rates of feeding (Cr), assimilation (Ar), production (Pr) and metabolism (Mr) are calculated by dividing C , A , P and R by the product of mid-body weight (g) of the insect and feeding duration (day)

$$Cr, Ar, Pr \text{ or } Mr = \frac{C, A, P \text{ or } R \text{ (J)}}{\text{Mid-body wt. (g)} \times \text{Feeding duration (day)}} \quad (7)$$

The procedure described above can also be followed for drawing the budgets for specific compounds or elements present in the food (Muthukrishnan and Pandian 1987). Besides mass and energy budgets, nitrogen and water budgets of insects are usually calculated. Energy budgets are useful to understand the adaptive strategies of organisms. Physiological trade-offs between Cr , Ae and Pe_2 in response to nutritional/environmental stress are not uncommon among phytophagous insects and they have helped them to achieve their ideal final body weight at an optimal growth rate.

2. Host plant parameters of bioenergetics significance

2.1 Nitrogen

Host plant characteristics vary with growth form of plants such as trees, shrubs, forbs or grasses. For leaf feeding insects, leaf chemistry rather than growth form of plant plays a decisive role. Of the many plant characteristics, nitrogen content is vitally important to herbivores. It ranges from 0.03–7% dry wt for different plants and averages 2.1% for 400 species of woody plants (Russell 1947). Within the same species, it differs significantly between seasons, ontogenetic stages, and tissues. During senescence, 20–40% decrease from the maximum level observed in the actively growing season has been reported (Mattson 1980). Tender leaf is richer in N than senescent leaf (e.g. tender *Ricinus communis* leaf 4.83% and senescent leaf 2.08%; Senthamizhselvan and Muthukrishnan 1989). Plant sap has less N (0.004–0.6%) than flowers (2–4%) or seeds (0.5–6.6%) (see figure 1A). C_4 grasses inhabiting xeric N poor soil have less N than the C_3 plants. Competition for the limited soil N between densely populated plants results in low N. Application of N fertilizer to soil increases leaf N by over 4 times. On the other hand, N content of herbivores including insects and mites is several times higher than that of plants and ranges from 7–14% (Scriber 1983). Therefore, N is a limiting nutrient for many phytophagous insects and increase in rates and efficiencies of ingestion and utilization of N may benefit them to realise optimum growth and reproductive success.

2.2 Water

For most terrestrial insects food is the major source of water. Water content of insects is usually higher than their host plants. For instance, most lepidopteran larvae maintain a water content as high as 85–92%. Maintenance of high water contents especially in desiccating terrestrial environment has been a challenging factor for most insects (Edney 1957). For a majority of phytophagous insects low leaf water content has been a major evolutionary hurdle (Southwood 1972). Leaf water

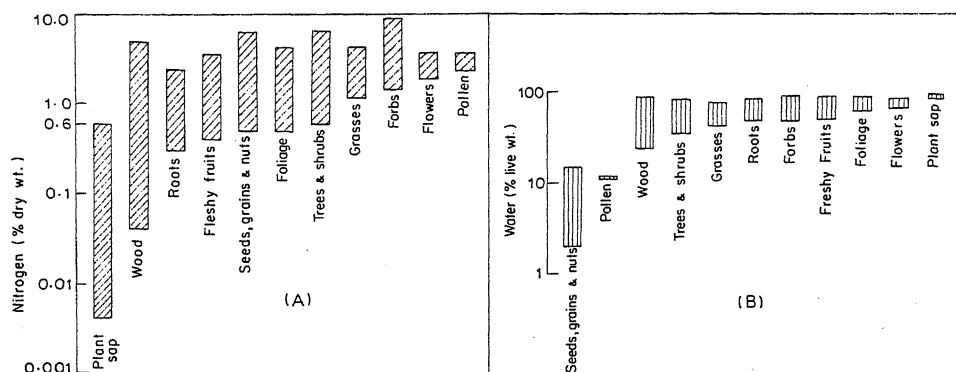


Figure 1. Nitrogen content (% dry wt) (A) and water content (% live wt.) (B) of host plants.

content covaries with N content in different growth forms, seasons and tissues of plants. It ranges from 75–95% in forbs, 60–85% in grasses and 45–75% in trees (figure 1B). It varies widely in young leaves compared with mature leaves.

2.3 Allelochemicals

These are chemicals evolved by plants to defend themselves against herbivores. However, some of them such as the calotropin of *Calotropis gigantea* have made them vulnerable to insect attack. Although they are considered as non-nutrients, some of them are utilized as nitrogen source by specialist insects (e.g. L-canavanine: Rosenthal *et al* 1982). Toxic amino acids, cyanogenic glycosides, alkaloids, toxic lipids, glucosinolates, sesquiterpene lactones and other terpenoids, saponins, phytohaemagglutinins, protease inhibitors, flavenoids, phenols, tannins and lignins, and insect hormones and antihormones are the major classes of allelochemicals (see Rosenthal and Janzen 1979). Broadly they are classified into two groups—qualitative chemicals which are susceptible to detoxification by specialist insects and quantitative chemicals such as fibers, lignins, silica, tannins, waxes and resins which are dose dependent and capable of reducing the digestibility of ingested food (Feeny 1976). Allelochemicals are capable of seriously impairing food utilization of phytophagous insects. Although nitrogen, water and allelochemicals of host plants are capable of independently influencing the rates and efficiencies of food utilization, it is difficult to quantify their independent influence. Fed on same host plant the performance of different species of insects significantly varies according to their life style, duration of larval development and final body weight attained. Similarly, performance of the same insect fed on different parts of the same host plant also significantly varies depending on the quality of the ingested parts.

3. Food consumption

Insects are capable of varying widely the total quantity of food consumed and the rate at which it is consumed. Besides host plant characteristics such as N, water and allelochemical contents, availability of the preferred host plant and the ability of insects to vary the final body weight are responsible for the observed variations in *C* and *Cr* of same species. Extensive experiments of Slansky and Feeny (1977) revealed that *Cr* of *Pieris rapae* fed on a variety of cultivated and wild plants with 2–5% of N varied from 80–40 J/mg/day. Their results showed a significant negative correlation between N content of host plant and *Cr* (figure 2). The chrysomelid beetle *Phaedon cochleariae* fed on 8-week old turnip leaf containing 49% less N than the leaf of the 4-week old plant displayed 28% increase in *Cr* over that feeding young leaf rich in N (Taylor and Bardner 1968). For the polyphagous *Pericallia ricini* fed on different host plants, Krishnan (1984) also obtained a negative correlation between *Cr* and N content of leaf.

On the other hand Senthamizhselvan and Muthukrishnan (1990) obtained a significant positive correlation between N content of *R. communis* leaf and *Cr* of *Ergolis merione* and *Porthesia scintillans* larvae (figure 3). They found an increase in the fertilizer (urea) dose applied to soil increased the N content of *R. communis* leaf and correspondingly the *Cr* of *E. merione* from 5.06–7.28 kJ/g/day and of

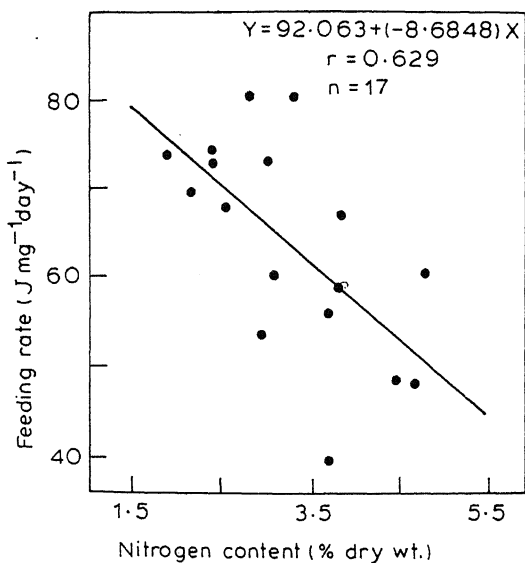


Figure 2. Feeding rate (Cr) of *Pieris rapae* as a function of N content of host plants (from Muthukrishnan and Pandian 1987).

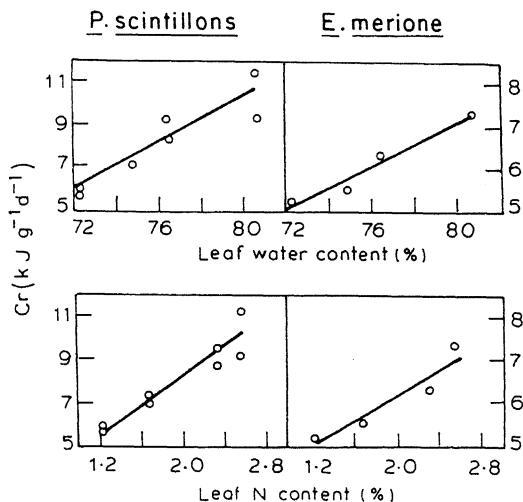


Figure 3. Feeding rate of *P. scintillans* and *E. merione* as a function leaf water and nitrogen contents (from Senthamizhselvan and Muthukrishnan 1990).

P. scintillans from 5.71–10.18 kJ/g/day. Faster Cr enabled the larvae to ingest more food. Similarly, higher N content of tender *Moringa olifera* leaf (4.83%) compared with that of senescent leaf (2.08%) doubled the Cr of *Eupterote mollifera* larva from 2.758–5.57 kJ/g/day (Senthamizhselvan and Muthukrishnan 1989). Penultimate instar *Papilio glaucus* larva feeding on forbs with 3% N content displayed 30% higher Cr (1.7 mg/mg/day) over that (1.31 mg/mg/day) feeding on tree foliage with 2.2% N (Scriber and Feeny 1979). Consideration of water content of the leaf in

addition to N content may explain the contrasting trends in *Cr* in relation to leaf N reported above. *R. communis* leaf raised on higher doses of fertilizer is not only rich in N but also water and energy contents (table 1). The tender *M. olifera* leaf contained more water (74.7%) and energy (20.06 J/mg) than the senescent leaf (water: 50.04%; energy: 14.95 J/mg) (Senthamizhselvan and Muthukrishnan 1989). Similarly the foliage of forbs contains more water than that of trees (figure 1B).

Pandian *et al* (1978) demonstrated the significance of leaf water content on feeding. They found that a minimum of 25% water content is obligatory to evoke feeding in the final instar *Danaus chrysippus* larva; 50% leaf water content enabled the larva to feed adequately to undertake pupation; 60% was the critical minimum water content for successful emergence. Corresponding to increase in the leaf water, *Cr* of the larva also increased. However, *Cr* of the IV instar *Hyalophora cecropia* larva fed on *Prunus serotinia* leaf with 70, 59, 55 and 49% water and 2.7–2.8% N content did not significantly vary (Scriber 1977). Scriber and Feeny (1979) proposed that N content of leaf is more important than water content for forb feeders, while water content is more important than N content for tree foliage feeding insects. Besides N and water, allelochemicals also significantly vary *Cr*, especially of polyphagous insects.

Whereas feeding as well as other bioenergetic parameters of a specialist insect are not seriously hampered by allelochemical with which it was associated during the coevolution, those of generalists widely fluctuate according to the nature and concentration of the allelochemical. *Cr* of *D. chrysippus* fed on *C. gigantea* leaf treated with up to 0.3% concentration of caffeine significantly decreased; further increase in the concentration to 0.5% resulted in an increase in the *Cr* (Muthukrishnan *et al* 1979). However, theophylline had a negative impact on *Cr* of *D. chrysippus*. Fed on *R. communis* leaf treated with 0.1, 0.3 and 0.5% ether extract of the neem kernal. Several plant chemicals possess antifeedant property. Flower extract of *Delonix regia*, seed extract of *Erythrina indica* and kernal extract of *Azadirachta indica* and tannic acid have been shown to deter feeding and decrease the *Cr* and *C* of many lepidopteran (Senthamizhselvan 1987) and coleopteran insects (Chandrakantha 1985). *R. communis* leaf treated with 0.5% ether extract of neem kernal decreased the *Cr* of *E. merione* to 0.9 kJ/g/day from 2.5 kJ/g/day of the larva feeding 0.1% extract treated leaf (table 2). Such plant chemicals seem to have a promising future as potential and safe pesticides.

Restricted availability of the preferred host plant due to over population of the pest and extensive defoliation elicits partial compensation by acceleration of *Cr* (Mathavan and Muthukrishnan 1976; Muthukrishnan *et al* 1978). For instance *Cr* of *Achaea janata* fed on 200 mg *R. communis* leaf/day was (3.98 kJ/g/day)

Table 1. Nitrogen, water and energy contents of the leaf of *R. communis* fertilized with different doses of nitrogen (urea).

Fertilizer (g/m ²)	Nitrogen (% dry wt.)	Water (% wet wt.)	Energy (J/mg)
75	1.235 ± 0.05	72.1 ± 3.40	18.568 ± 1.32
150	1.758 ± 0.06	74.8 ± 3.60	19.145 ± 1.40
300	2.322 ± 0.07	76.4 ± 3.9	22.122 ± 1.30
400	2.662 ± 0.06	80.7 ± 4.1	22.926 ± 1.32

Table 2. Bioenergetics of final instar *E. merione* fed *ad libitum* on *R. communis* leaf treated with different concentrations of ether extract of *A. indica* seed.

	<i>A. indica</i> seed extract concentration		
	0.1%	0.3%	0.5%
<i>D</i>	3.0	3.5	4.5
<i>C</i>	1834	1379	1021
<i>FU</i>	1054	845	686
<i>A</i>	780	534	335
<i>P</i>	339	183	109
<i>R</i>	441	351	175
<i>Cr</i>	2.5	1.1	0.9
<i>Ar</i>	1.1	0.4	0.3
<i>Pr</i>	0.5	0.1	0.1
<i>Mr</i>	0.6	0.3	0.2
<i>Ae</i> (%)	42.5	38.7	32.8
<i>Pe</i> ₁ (%)	18.5	13.2	10.7
<i>Pe</i> ₂ (%)	43.5	34.2	32.6

C, *FU*, *A*, *P* and *R* in J/insect.

Cr, *Ar*, *Pr* and *Mr* in kJ/g/day.

proportionately 22% higher than that (5.52 kJ/g/day) fed on two times more food (400 mg/day) (Muthukrishnan and Pandian 1984). Fed on restricted ration (25% of *ad libitum* level), the grasshopper *Poecilocerus pictus* extended the nymphal duration and undertook an additional moult in order to acquire as much food energy as possible (Muthukrishnan and Delvi 1974). It may be interesting to note that insects feeding on same host plant display significantly different *Cr*. For instance fed on *C. gigantea*, *P. pictus* ingested it at the rate of 2.2–2.3 kJ/g/day compared with 17.7–18.1 kJ/g/day of *D. chrysippus* (table 3). Such differences may be explained by the life style of the insects. To compensate the non-feeding pupal and adult stages, the holometabolous *D. chrysippus* feeds at a faster rate than the hemimetabolous *P. pictus* which feeds throughout life (see Pandian 1973). Among the lepidopteran larvae moths are reported to feed at a faster rate than butterflies (table 3). It may be concluded that besides host plant characteristics, availability of the host plant, life stage and style of the insect determine the *Cr*.

4. Assimilation

Assimilation (*A*) and the rate of assimilation (*Ar*) are (i) directly influenced by the spectrum of digestive enzymes secreted, their rate of secretion and activity and (ii) indirectly influenced by *Cr*. Host plant characteristics such as N, water and allelochemic contents also influence assimilation indirectly through *Cr*. Allelochemicals also exert a direct influence on assimilation efficiency by their effect on digestive enzymes. As the size of the meal passing through the foregut triggers secretion of digestive enzymes and determines their activity, a constant fraction of the ingested food is assimilated (Muthukrishnan and Pandian 1987).

Pandian and Marian (1986) related the *Ae* of over 60 species of lepidopteran larvae ranging from 19–81% with the N and water content of host plants and

Table 3. Inter specific differences in the utilization of *C. gigantea* leaf by the grasshopper *P. pictus* and the butterfly *D. chrysippus* and *R. communis* leaf by the butterfly *E. merione* and the moth *S. exigua*.

	<i>P. pictus</i> ^a		<i>D. chrysippus</i> ^b		<i>E. merione</i>	<i>S. exigua</i>
	0	0	0	0		
<i>D</i>	150.0	147.0	9.0	9.0	9.0	7.5
<i>C</i>	621.18	276.78	11.74	11.34	11.91	12.88
<i>FU</i>	282.91	120.40	5.53	4.86	5.72	6.88
<i>A</i>	338.27	156.38	6.21	6.48	6.19	6.00
<i>P</i>	171.84*	17.46	2.63	2.39	2.14	1.76
<i>R</i>	166.43	138.92	3.58	4.09	4.05	4.24
<i>Cr</i>	2.30	2.20	17.69	18.11	14.82	18.75
<i>Ar</i>	1.35	1.24	10.62	11.77	8.54	9.85
<i>Pr</i>	0.64	0.14	4.88	4.71	1.19	1.97
<i>Ae</i> (%)	54.5	56.5	52.9	57.1	52.07	46.6
<i>Pe</i> ₁ (%)	27.6	6.3	22.4	21.1	18.0	13.7
<i>Pe</i> ₂ (%)	50.8	11.2	42.4	36.9	34.6	25.6

^aRecalculated from Delvi (1972); rearing temperature 36°C.

^bRecalculated from Mathavan and Bhaskaran (1975); rearing temperature 32°C.

*Includes egg production of 46.93 kJ.

D, Feeding duration; *C*, consumption; *FU*, faeces and urine; *A*, assimilation;

P, production; *R*, metabolism (in kJ/insect) *Cr*, *Ar*, *Pr* rates of *C*, *A*, and *P* in kJ/g insect/day.

obtained a highly significant positive correlation between food N and *Ae* ($r=0.704$). The relationship was more significant ($r=0.868$) when water content of the host plants was considered as a second predictor variable. However, increase in N and water contents of *R. communis* leaf due to increased fertilizer application decreased the *Ae* of *E. merione* and *P. scintillans* (Senthamizhselvan and Muthukrishnan 1990).

Digestibility reducing quantitative chemicals such as tannins, lignins and fibres increase in concentration with age of the host plant. Therefore, earlier instars which prefer to feed on tender leaf with more N and water and less concentration of digestibility-reducing chemicals assimilate the food more efficiently than the later instars which feed tough senescent leaf (Senthamizhselvan and Muthukrishnan 1989). Some of the chemicals like tannin bind with leaf protein and render the plant tissue only partly digestible (Denno and Donnelly 1981). A few of them like soyabean trypsin inhibitor bind with proteases and render them inactive (Applebaum and Brick 1972). Effect of digestibility-reducing chemicals of the host plant varies between species. Grasshoppers and beetles display high tolerance to lignin and tannin compared with lepidopteran larvae (Bernays 1978). Effect of qualitative chemicals such as gallic acid, flavonoids, terpenoids etc. is mostly concentration-dependent and varies also with age of the insect. For instance, with increasing gallic acid concentration from 0.01–0.05 and 0.1 M in the leaf, *Ae* of *Spodoptera littoralis* decreased from 52–38 and 33%, respectively (Mansour 1981). *Ae* of *E. merione* fed on 0.5% neem extract treated leaf was (32.8%) significantly less than that (42.5%) fed on 0.1% extract treated leaf (table 4). Whereas the efficiency of final instar *Spodoptera eridania* decreased with the concentration of DIMBOA in the maize that of the penultimate instar increased (table 4). On the other hand caffeine (0.5%) increased the *Ae* of *D. chrysippus* (Muthukrishnan *et al* 1979).

Table 4. Effect of plant allelochemicals on assimilation efficiency ($X \pm SD$) of Lepidopterous larvae (from Muthukrishnan and Pandian 1987).

Chemical and its concentration	Assimilation efficiency (%)	Reference
<i>A. ipsilon</i> (V instar)		
Control	34 ± 1.1	Reese and Beck (1976)
<i>p</i> -Benzoquinone, 3.75 × 10 ⁻² M	26 ± 2.2	
Control	31 ± 0.7	
Duroquinone, 3.75 × 10 ⁻² M	28 ± 0.9	
Control	41 ± 1.6	
Resorcinol, 3.75 × 10 ⁻² M	45 ± 3.4	
Control	32 ± 0.5	
Phloroglucinol, 3.75 × 10 ⁻² M	30 ± 0.5	
Control	38 ± 1.5	
Gallic acid, 3.75 × 10 ⁻² M	29 ± 1.7	
<i>S. littoralis</i> (V instar)		
Control	51	Mansour (1981)
Gallic acid, 1 × 10 ⁻² M	52 ± 1.2	
5 × 10 ⁻² M	38 ± 0.8	
10 ⁻¹ M	33 ± 1.9	
Coumarin, 1 × 10 ⁻¹ M	Refused to feed	
1 × 10 ⁻² M	56 ± 0.7	
5 × 10 ⁻² M	44 ± 0.5	
<i>S. eridania</i> (V instar)		
B-49 corn DIMBOA, 5.5 mg g ⁻¹	81 ± 0.7	Manwoto and Scriber (1981)
San Juan corn DIMBOA, 3.59 mg g ⁻¹	79 ± 2.8	
DIMBOA-free corn	68 ± 2.5	
DIMBOA 2.4 mg g ⁻¹		
<i>S. eridania</i> (VI instar)		
DIMBOA, 5.5 mg g ⁻¹	58 ± 5.0	Manwoto and Scriber (1981)
3.59 mg g ⁻¹	61 ± 3.8	
2.45 mg g ⁻¹	64 ± 2.8	
<i>D. chrysippus</i> (V instar)		
Control	58 ± 3.0	Muthukrishnan <i>et al</i> (1979)
0.5% caffeine	70 ± 1.0	

It is generally believed that *Ae* is negatively correlated to *Cr*. A faster *Cr* results in rapid passage of food through the gut and provides less time for digestive enzymes to act on the substrate. Increase in *Cr* of *E. merione* and *P. scintillans* feeding N and water-rich leaf account for the decrease in *Ae* (Muthukrishnan and Senthamizhselvan 1990). Decreased consumption of the less preferred host plant was compensated by increased assimilation efficiency *S. eridania* (Soo Hoo and Fraenkal 1966). The relationship between *Cr* and *Ae* is a complex one. A low *Cr* may increase the *Ae* because of prolonged retention of food in the gut and a highly digestible food may lower the *Cr*. Therefore, it is difficult to evaluate the cause and effect relationship between them.

5. Metabolism

Part of the assimilated energy is allocated to metabolism. It depends on that quantum of ingested energy available for assimilation and required for growth to the critical minimum weight. In addition to maintenance of vital activities, the very act of feeding involves expenditure of energy. Foraging insects like bees spend 3.7–8.2% of the energy of the pollen and nectar collected by them as energy cost of foraging (Pandian 1985). Energy allocated to metabolism bears a significant positive linear relationship with Cr in several insects (Pandian *et al* 1978; see also Muthukrishnan and Pandian 1987). Increase in Cr over this minimal level involves further expenditure of energy. To enhance the Cr by 1 J/g/day above the maintenance level, *A. janata* reared at 27°C has to spend 0.225 J/g/day on metabolism (Muthukrishnan and Pandian 1984).

Low water content and high concentrations of allelochemicals in the food result in enhanced metabolic rate. Insects feeding on wilting senescent leaves (Marian and Pandian 1980) and those feeding tree leaves (Scriber 1978) with low water content suffer considerable metabolic strain on account of producing metabolic water. Detoxification of toxic chemicals in the host plants also imposes a heavy metabolic cost. Phytophagous insects employ mixed function oxidases (MFO) for this purpose. Unfortunately the metabolic cost of secreting and operating MFO has not been worked out so far. However it can be inferred from the data on net conversion efficiency (Pe_2) of insects reared on host plants with different concentrations of toxins (table 5). Detoxification of 0.5% nicotine or 0.1% atropine in the host plant cost 8 or 3% of assimilated energy for the final instar *Manduca sexta* larvae. Ingestion of 0.5% caffeine treated leaf doubled the metabolic cost of *D. chrysippus* larva (Muthukrishnan *et al* 1979). However, Mr of *E. merione* feeding 0.5% neem extract treated leaf was 3 times less than that feeding 0.1% extract treated leaf (table 2). Manuwoto and Scriber (1981) have shown that previous experience with the plant toxin would help the insect to considerably minimise the energy expenditure on detoxification. Exposure to unusual toxins as experienced by the insects while switching over to unusual host plants enhances the cost of detoxification, as the insect has to secrete an appropriate enzyme for the purpose.

6. Conversion

Percentage of assimilated energy allocated to tissue growth (Pe_2) varies widely among phytophagous insects. For instance, it varies from 2–87% in the for-chewing Lepidoptera, 8–72% in the tree foliage feeding Coleoptera and 8–54% in the grass feeding Orthoptera (Slansky and Scriber 1982). Host plant quality especially N, water and allelochemic concentrations account for such variations in Pe_2 . Depending on host plant quality polyphagous insects adjust the rate of food consumption (Cr) and efficiencies of assimilation (Ae) and growth (Pe_2). For instance, the final instar *P. rapae* adjusts the high N content (6.9%) of *Brassica oleracea* and the consequent increase in Cr to 4.232 kJ/g/day by maintaining the Ae (28%) and Pe_2 (41.9%) at low levels; on the other hand, feeding N-poor (3.83%) *Cleome spinosa*, the larva maintains a low Cr (3.136 kJ/g/day) but assimilates (Ae = 33%) and converts (Pe_2 = 54.5%) with greater efficiency (Slansky and Feeny 1979). Finke (1977) has also reported a positive relation between food N and gross

Table 5. Effect of plant toxins on the net conversion efficiency (Pe_2) of final instar lepidopterous larvae as an indication of the cost of neutralization of toxins (from Muthukrishnan and Pandian 1987).

Species	Test food	Pe_2 (%)	Reference
<i>M. sexta</i>	Diet	48.5	Schoonhoven and Meerman (1978)
	Diet + 0.5% nicotine	40.3	
	Diet + 0.1% atropine	45.5	
<i>D. chrysippus</i>	<i>C. gigantea</i>	48.7	Muthukrishnan <i>et al</i> (1979)
	+ 0.1% caffeine	42.6	
	+ 0.2% caffeine	35.3	
	+ 0.3% caffeine	32.0	
	+ 0.4% caffeine	27.9	
	+ 0.5% caffeine	18.0	
	+ 0.1% theophylline	43.6	
	+ 0.2% theophylline	44.5	
	+ 0.3% theophylline	39.7	
	+ 0.4% theophylline	35.0	
	+ 0.5% theophylline	28.6	
<i>S. littoralis</i>	Semisynthetic diet	44.2	Mansour (1981)
	+ 10^{-2} M gallic acid	39.5	
	+ 10^{-1} M gallic acid	38.3	
	+ 10^{-2} M coumarin	18.0	
<i>S. eridania</i> ^a	Low DIMBOA corn ^b (0.05 mg DIMBOA day ⁻¹)	53.6	Manuwoto and Scriber (1981)
<i>S. eridania</i>	San Juan corn (0.1 mg DIMBOA day ⁻¹)	17.3	
	B49 corn (0.23 mg DIMBOA day ⁻¹)	12.4	

^aPenultimate instar. ^b2,4-Dihydroxy-7-methoxy-1,4-benzoxazine.

conversion efficiency (Pe_1) of *Papilio polyxenes* larva. A direct linear relationship between leaf water content and conversion efficiency (Pe_1 and Pe_2) has been reported for the polyphagous *S. eridania* by Soo Hoo and Fraenkel (1966) and *H. cecropia* by Scriber (1977). An increase in leaf water content from 52–90% resulted in an increase in the Pe_2 of lepidopteran larva from 8–50% (Scriber 1979). As stated already energy cost of production of metabolic water or detoxification of defense chemicals results in low Pe_2 . Any factor which decreases energy expenditure on metabolism would help the insect to enhance the production efficiency. The sawfly *Neodiprion sertifer* larva restricts its active metabolism to a low level by avoiding movement in search of feeding sites and manages to realise a Pe_2 as high as 81%.

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Insect induced plant galls in tissue culture

U KANT and VIDYA RAMANI

Plant Pathology and Tissue Culture Laboratory, Department of Botany, University of Rajasthan, Jaipur 302 004, India

Abstract. Plant tissue culture enabling the assessment of excised organ tissues and cells and the effect of various metabolites and gall and normal tissues are discussed with reference to insect induced plant galls. In particular the stem and rachis galls of *Prosopis cineraria* caused by a chalcid and *Lobopteromyia prosopidis*, stem gall of *Embllica officinalis* Gaertn induced by a Lepidopteran, *Betousa stylophora* Swinhoe, stem gall of *Zizyphus mauritiana* induced by a mite, *Eriophyes cernuus*, the *Phylloxera* gall on grape and many others are discussed.

Keywords. Plant galls; tissue culture; *Prosopis cineraria*; *Lobopteromyia prosopidis*; *Embllica officinalis*; *Betousa stylophora*; *Zizyphus mauritiana*; *Eriophyes cernuus*; *Phylloxera*.

1. Introduction

Galls are manifestations of growth, positive or negative and of abnormal differentiation induced on a plant by animal or plant parasites (Meyer 1987). They may arise on shoots, roots, petioles, stipules, leaf-blades, vegetative and flower buds, inflorescence axis, bracts, flowers, fruits, etc. Agencies causing galls include physical and chemical agents, genetic constitution, bacteria, viruses, fungi, nematodes, insects and mites.

Although excellent reviews have been contributed in the field of gall induction (Miles 1968), ecology (Mani 1964, 1973) and gall histopathology (Rohfritsch and Shorthouse 1982), the factors involved in gall development need satisfactory answers. Certain critical questions involved in tumor induction with reference to nature of incitant, tissues affected, the stimulus that keeps them growing and the mechanism by which the abnormal growth can be stopped, remain to be answered. According to Norris (1979) insects probably utilise their own specific and general chemicals in the induction of a gall.

Tissue culture technique has been used in a variety of ways in physiological, cytological, biochemical and morphogenetic problems of plants. The *in vitro* culture of tissues provides a system in which many variables can be controlled, enabling the assessment of excised organs, tissues and cells. Plant tissue culture offers certain advantages over the intact plant as an experimental material (Hildebrandt and Riker 1947; Bouckaert and Vendrig 1981).

The basic idea of tissue culture was conceived in the beginning of the 20th century. Haberlandt (1902), a German botanist has been credited with putting the technique into action. The continuous growth of isolated root tips for unlimited periods was successful first with the work of White (1939). Subsequently, successful callus cultures were established from carrot by Gautheret (1939) and Nobecourt (1939) and from tobacco by White (1939) independently.

Among the first tissues grown in culture was the genetic tumor tissue of *Nicotiana glauca* hybrid (*Nicotina glauca* × *N. langdorffii*) (White 1939). Extensive studies

have been made with this tissue culture, including work on tumorigenesis and fine structure (Skoog 1944; Chilton *et al* 1977). Gall tissue of unknown origin on white spruce was isolated and studied (Reinert and White 1956). Tissue of normal and disease origin may now be grown for several years by regular transfer to fresh medium.

2. Tumor tissues in culture

The use of plant tissues to study tumorigenesis complements investigations of transformed animal cells. Moreover, because plant cells are usually totipotent, the important phenomenon of reversal of tumorigenesis may be investigated most readily in plants. In addition to the bearing on the cancer problem, plant tumor experimentation promises to yield information on the control mechanisms that restrain and regulate growth of normal plant tissues (Gordon 1981).

Gautheret (1955) has noted that when normal cells are grown in culture some cells spontaneously grow without exogenously supplied hormones. Such 'habituated tissues' may be isolated from a variety of plant species. Since the phytohormone content of these tissues is of the same order of magnitude as in some crown gall tissues, it seems that the galls have the capacity to produce these materials (Kulescha 1952). Thus, these cells express one of the traits characteristic of crown gall tumor tissue. It is also possible to obtain cultures that are auxin or cytokinin habituated. Treatment with animal carcinogens cause tobacco tissues to lose their requirement for cytokinin (Bednar and Linsmair-Bednar 1971). Thus it is possible to switch on and switch off the biosynthetic pathways leading to the synthesis of phytohormones in non-tumorous tissues.

Reviews covering the state of plant tumor field before the recent flurry of plasmid DNA oriented work can be found in Schilperoort (1969). An important review in the field before the 1970s is found in a monograph edited by Braun (1972). More recent developments have been reviewed by Lippincott and Lippincott (1980), Schell and Van Montagu (1978) and David and Tempe (1987).

A few studies have also been made with viral tumor tissue cultures. Black (1949) isolated tissues from root tumors of *Rumex acetosa*. Detailed nutritional and other studies with virus tumor tissue have been done (Nickell and Burkholder 1950).

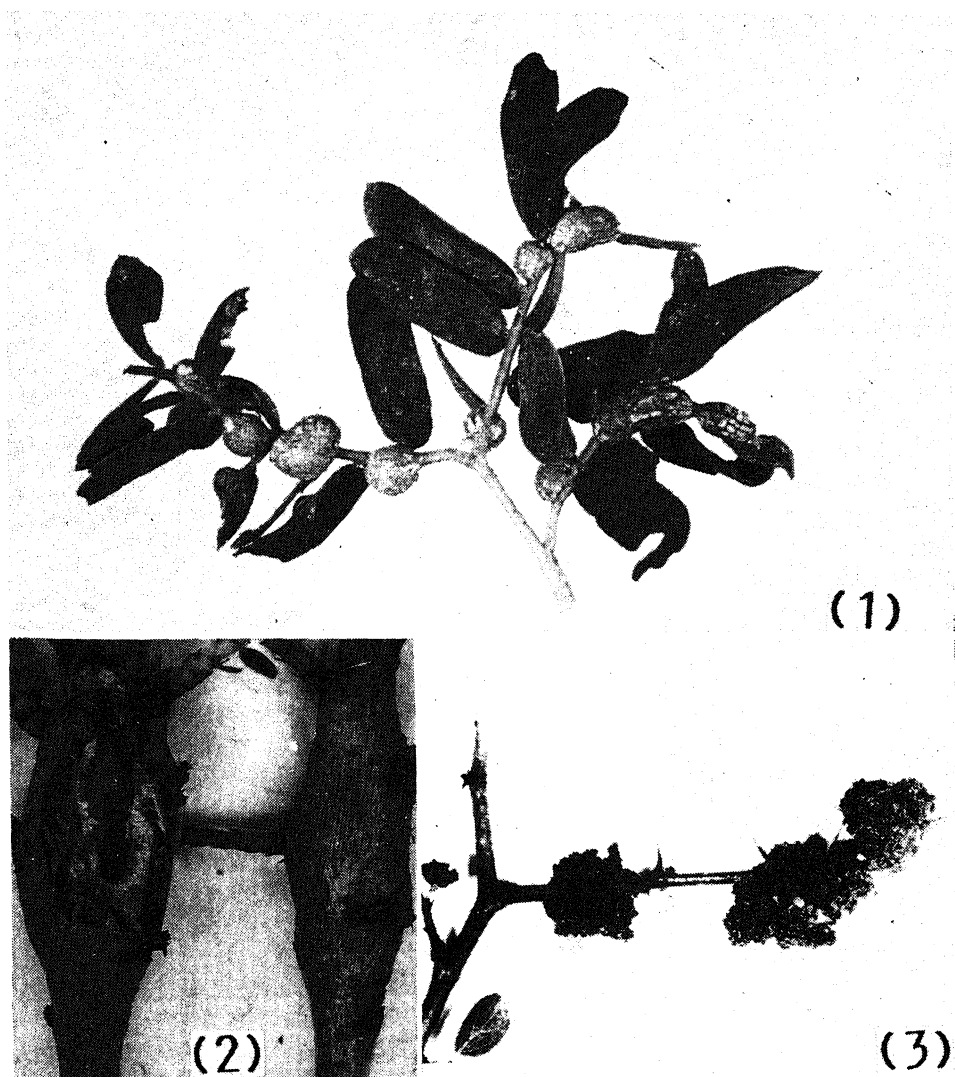
Suspension culture was established many years after the establishment of static culture (Muir *et al* 1954). Single cells were grown and single cell clones were established (Muir *et al* 1958). Growth and tissue formation from single geranium cells and from virus infected geraniums have been studied (Kant and Hildebrandt 1971). Isolation, culture and fusion of protoplasts have lately been possible (Cocking 1960; Street 1973). The composition of the medium is critical for growth. Earlier work with tissue culture provided valuable information regarding media employed and types of tissues and organs cultured. The work has been reviewed in detail (Gautheret 1955; Street 1973).

3. Culture of insect gall tissues

A number of insect-induced gall tissues have been isolated and successfully cultured *in vitro*. Many insect-induced plant gall tissues especially from woody plants were

successfully cultured (Hildebrandt *et al* 1956; Arya *et al* 1962a, b; Arya 1965a, b; Kant and Arya 1969; Kant and Singh 1976; Arora and Kant 1979; Kant 1985).

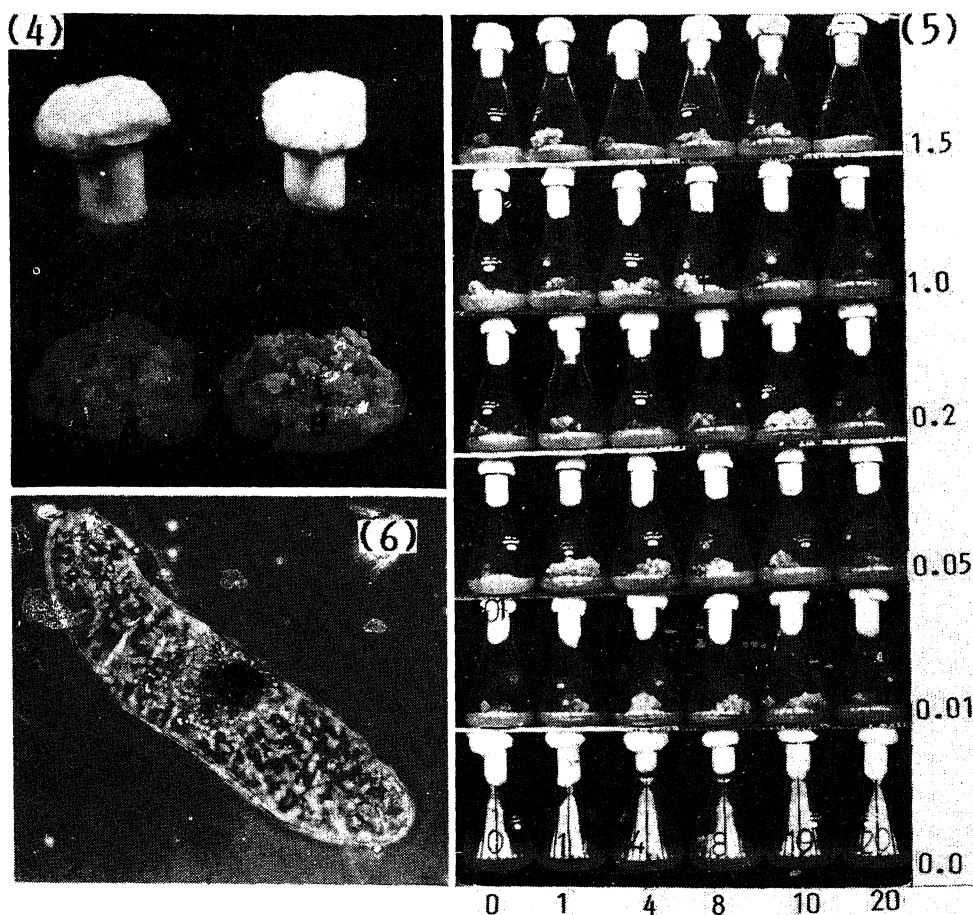
It has been shown that insect-induced plant gall tissues grew better on media supplemented with coconut-milk or coconut-milk with 2,4-dichlorophenoxy acetic acid (2, 4-D) and α naphthalene acetic acid (NAA) (Hildebrandt *et al* 1956). Nutritional and other requirements of *Phylloxera* gall and normal grape stem tissue have been examined in detail (Pelet *et al* 1960; Arya 1965a, b). Excellent work with single cell clones of *Phylloxera* gall and normal grape stem tissue have been done (Arya 1965a, b). Studies related to nutrition and growth have been made for normal and gall tissues of *Zizyphus jujuba* Lamk. (Kant and Arya 1969; Vyas 1971; Tandon *et al* 1976). Tandon *et al* (1976) have studied some aspects of gall induction on *Zizyphus*. Kant



Figures 1-3. 1. Rachis gall of *P. cineraria* induced by *L. prosopidis*. 2. Stem gall of *E. officinalis* induced by *B. stylophora*. 3. Stem gall of *Z. mauritiana* induced by *E. cernuus*.

and Singh (1976) have studied the effects of vitamins, choline chloride on gall and normal tissues of *Z. jujuba*. Studies related to nutrition and growth have been made for gall and normal tissues of *Emblica officinalis* (Arora and Kant 1979).

Kant (1967) studied the morbid anatomy of some insect induced galls, common to Rajasthan. The cultures of *Z. jujuba* gall and normal tissue were established on a semisynthetic medium (Kant and Arya 1969). Subsequently, cultures of *Zizyphus* gall and normal tissue were established on a synthetic medium (Murashige and Skoog 1962) by Vyas (1971). Basic physicochemical, carbohydrate, nitrogen, vitamin and growth factor requirement of normal and gall tissues in culture were determined. Arora and Kant (1979) have studied the physiology of *E. officinalis* normal and gall tissues in culture. Singh (1978) observed the radiation effects on *Zizyphus* gall and normal tissues and cells. Ranwa (1983) has carried out further studies on the physiology and biochemistry of gall and normal tissues of *E. officinalis*. Chatterjee



Figures 4-6. 4. Normal and gall callus of *E. officinalis*. (A) Normal callus, (B) Gall callus. 5. IAA-kinetin interaction of gall callus tissue of *P. cineraria*. From left to right increasing concentrations of IAA in mg/l. From bottom to top increasing concentrations of kinetin in mg/l. 6. A single cell of gall callus of *P. cineraria*. ($\times 400$).

(1984) has studied stem gall of *Prosopis cineraria* in culture. Kant and Ramani (1988a) have carried out *in vitro* studies on rachis gall of *P. cineraria*.

Most of the work on galls have been carried out in economically important plants and some semi-arid zone trees. Galls studied *in vitro* include the stem and rachis gall of *P. cineraria* (a tree of the semi-arid zone) induced by a chalcid and the insect *Lobopteromyia prosopidis* Mani respectively; the stem gall of *E. officinalis* induced by *Betousa stylophora* Swinhoe, the stem gall of *Zizyphus mauritiana* Lamk. induced by *Eriophyes cernuus*, a mite. The following important aspects of these galls have been dealt with:

- (i) Isolation of normal and gall tissues and their establishment on a suitable medium.
- (ii) Carbohydrate and nitrogen metabolism of gall and normal tissues.
- (iii) Auxin, cytokinin and phenol metabolism of tissues.
- (iv) Vitamin metabolism.
- (v) Cytomorphological studies of gall and normal tissues.
- (vi) Biochemical studies *in vitro* (and *in vivo*).

3.1 Isolation and establishment of normal and gall callus

Isolation of gall callus was carried out in the following manner. Young galls were longitudinally split into two and the insect was removed. Split gall pieces were treated with mercuric chloride solution (0.1%) for 15–20 min. Sterilized gall pieces were maintained and subcultured on suitable media (generally MS medium) supplemented with growth regulators. Similarly, the normal callus was isolated from the normal counterpart, and maintained on MS medium.

3.2 Nutritional studies

Nutritional studies revealed differential behaviour of normal and gall tissues to various sources of carbohydrates, nitrogen, vitamins, auxin, cytokinin and phenolics. Very little growth of tissue was recorded in the absence of these metabolites. Auxin-kinetin interaction has also been studied (Kant and Ramani 1987). Partial cytokinin autotrophy has been recorded for the rachis gall tissues of *P. cineraria*. The effect of various phenolic substances viz. *t*-cinnamic acid, ferulic acid, *p*-hydroxybenzoic acid, caffeic acid, catechol etc. on tissue growth has been studied. None of the phenolics were essential for tissue growth, although very low concentrations (0.1–1.0 mg/l) of cinnamic acid sustained good growth of gall tissue. *p*-hydroxybenzoic acid and catechol inhibited growth of tissues.

3.3 Cytomorphological studies

Cytomorphology of normal and gall callus cells was carried out by the microculture chamber method of Jones *et al* (1960). The microculture chamber method has made it possible to isolate and examine, living cells of higher plants, in an aseptic slide culture. Under sterile conditions, two drops of mineral oil were spaced on either side of the slide and sterilized cover slips placed on each drop to provide two risers to

support a third cover slip over the cell culture. A drop of the liquid medium suspension of cells (gall and normal on separate slides) was placed between the two risers, a mineral oil dam added around the cell suspension, and a third sterile cover slip laid on the two risers and over the cell suspension. Single cells may also be isolated and grown in the microchamber. By this method, cytomorphology of normal and gall callus cell was studied under a phase contrast microscope. It revealed differences in the relative percentages, shapes and sizes of cells at different phases of tissue growth, between normal and gall tissues. Hypertrophied nuclei and cellular hyperplasy were evident in the gall callus cells of these plants (Chatterjee 1984).

3.4 Isolation of single cell clones

Clones of tissues may be established from single cells isolated from callus cultures of higher plants using a filter paper-nurse culture method (Muir *et al* 1954). A small piece of sterile filter paper was first placed on an established piece of nurse tissue growing in a flask for two or three days until it had become moistened by the nutrients from the host tissue piece. The piece of filter paper was then placed in a sterile petri plate on agar medium besides a spread suspension of cells or a callus piece. A single cell was then selected under the binocular microscope and transferred to the filter paper and then the filter paper with the cell returned to the nurse-piece in the culture flask. The single cell or the resulting cell mass may be examined for growth at any interval by removing the filter paper with its cell or cells to agar in a petriplate under a microscope. It may be necessary to transfer the filter paper with the small group of cells to several fresh nurse-pieces as the nurse-piece ages or becomes dry. Eventually, a mass of cells of sufficient size develops that will grow when transferred directly to the agar medium. This constitutes a clone of tissue of single cell origin. This method has opened new areas for study of tissues of normal and diseased (or galled) origin at the cellular level. Single cell clones of many plants have been established. *Phylloxera* (gall) single cell clones have been established as mentioned earlier.

3.5 Biochemical studies

Biochemical studies revealed in general, that the gall tissue contained more carbohydrates and reducing sugars. Gall tissues of *Z. mauritiana*, *E. officinalis* and stem gall tissue of *P. cineraria* have revealed hyperauxiny (Kant and Ramani 1988a,b) with the exception of rachis gall of *P. cineraria* (Ramani *et al* 1989). Changes in the activity of various enzymes viz. amylase, indole-3yl acetic acid (IAA) oxidase, phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), peroxidase, and polyphenol oxidase have also been recorded. While the gall tissues showed a marked decrease in IAA oxidase, and polyphenol oxidase activities, with varied activity of peroxidase (Kant and Ramani 1988a, b), high amount of total phenolics and O-dihydroxy phenols were recorded in the gall tissues. Unregulated synthesis of auxin protectors (O-dihydroxy phenol) have been held responsible for hyperauxiny. These substances prevent IAA destruction by inducing a lag period in the oxidation of IAA, thus causing hyperauxiny and abnormal proliferation of the gall tissue.

Role of oxidative enzymes and phenolics in hyperauxiny and abnormal growth in many other insect and mite induced galls such as *Achyranthes aspera* (Shekhawat *et al* 1978), *Cordia myxa* (Ramawat *et al* 1979), *Camellia sinensis* and *Elaeocarpus lancifolius* (Joshi *et al* 1985) have been established.

4. Conclusion

Most of the galls studied have shown increased production of growth hormones, particularly auxin and cytokinin in diseased cells. The tumefacient properties reported for *Zizyphus* gall tissue incited by a plant mite (*E. cernuus*) has given new dimensions to the problem of gall formation in plants. Crucial research needs to be directed towards understanding the molecular mechanism of insect and mite induced gall formation in plants.

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Systematics and speciation in relation to insect-plant interactions

K G SIVARAMAKRISHNAN

Department of Zoology, Madura College, Madurai 635 011, India

Abstract. Problems of systematics and speciation in the context of recent advances in the field of insect-plant interaction are highlighted with reference to the comparative study of speciation in relation to the population structure and genetic architecture of living organisms. The role of insect-plant interaction in the biosystematic study of some aquatic weevils, aphids and archid pollinators are also discussed besides sympatric speciation in some phytophagous insects.

Keywords. Insect-plant interactions; biosystematics; population structure; genetic architecture; sympatric speciation; phytophagous insects.

1. Introduction

Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them. The word 'relationship' is not used in a narrow phylogenetic sense, but is broadly conceived to include all biological relationships among organisms. One of the major preoccupations of systematics is to determine, by comparison, what the unique properties of every species and higher taxon are. Another is to determine what properties certain taxa have in common with each other, and what the biological causes for the differences or shared characters are. Finally, it concerns itself with variation within taxa. Biosystematics, in particular is given the rightful importance among biological disciplines, more because of increasing need for a correct determination of generic and specific complexes, in view of the occurrence of large-scale diversities within species which make bioecological, behavioural as well as related interdisciplinary approaches obligatory for a better understanding of these complexes (Ananthakrishnan 1987). Furthermore, modern accounts of organic evolution recognize the existence of two processes: anagenesis, phyletic change in the course of geological time; and cladogenesis or speciation, the origin of new species of organisms through splitting of pre-existing ones. Both are population phenomena. Cladogenesis compensates for the loss of species by extinction, so that no sudden or major changes in the total amount of biotic diversity occur. It is becoming more and more apparent that speciation can occur in different ways (Dobzhansky *et al* 1977). Thus, the comparative study of speciation, in relation to the population structure and genetic architecture of living organisms, is assuming an increasing importance in evolutionary studies. The aim of this review is to highlight the trends in our present understanding of the problems of systematics and speciation in the context of recent advances in the field of insect-plant interactions.

2. Biosystematics of aquatic weevils infesting water weeds

A typical instance of the role of insect-plant interaction in the biosystematic study of two species of aquatic weevils will highlight the significance of such studies in

future. Biological control of the aquatic weed, *Salvinia molesta* was first attempted in southern Africa with the introduction of the weevil, *Cyrtobagous singularis* from Trinidad which established (Procter 1984) but failed to control the weed in the upper Zambezi or Chobe rivers (Edwards and Thomas 1977). A closely related weevil, *C. salviniae* from Brazil, has proven to be a most effective agent for *S. molesta* in northern Australia (Room *et al* 1981), in Papua New Guinea (Thomas 1985) and in Bangalore (Jayanth 1987). *C. salviniae* was first thought to be a biotype of *C. singularis* specially adapted to *S. molesta* (Forno *et al* 1983) but was later shown to be a separate species (Calder and Sands 1985). This was substantiated by the studies of Sands and Schotz (1985), May and Sands (1986) and Sands *et al* (1986) contributing to the different impact on the weed by the two species.

2.1 Differences in feeding strategies

Damage by *C. salviniae* to *S. molesta* results from larvae tunnelling within the rhizome (Sands *et al* 1983), a feeding behaviour different from *C. singularis* whose larvae feed externally on submerged parts of the plant. It is possible that the longer setae of the larvae of *C. singularis* are an adaptation to external feeding, where they aid movement from one submerged part of the host to another, in contrast to the shorter setae of *C. salviniae* whose larvae tunnel internally within the rhizome (May and Sands 1986). Adult behaviour also differs, *C. salviniae* feeding mainly on buds of *S. molesta* whereas *C. singularis* feed extensively on other parts of the plant leaving more buds intact. Hence, *C. singularis* has less impact than *C. salviniae* on plant growth (Sands and Schotz 1985).

2.2 Differences in preoviposition period, oviposition period, the pattern of oviposition and in effects of plant nitrogen on fecundity

The pre-oviposition and oviposition periods are very variable for both species. The pattern of oviposition was markedly different for the two species, *C. salviniae* laying eggs almost continuously (92% of weeks with some eggs) while *C. singularis* laid eggs intermittently (50% of weeks with eggs). Concentration of N in the food plant had greater effect on oviposition by *C. singularis* when compared to that of *C. salviniae*. The values for the intrinsic rate of increase (r_m) suggest that *C. salviniae* is physiologically better adapted to *S. molesta* than *C. singularis*, particularly in the higher developmental temperature range. The differences are likely to be accentuated by the greater dependence of *C. singularis* on N in the host plant, which may be due in part, to its adaptation to a different host, *S. auriculata*, a plant found to concentrate 0.4% more N than *S. molesta* when grown in a nutrient containing 3 mg/l of N (Sands *et al* 1986). Thus, differences in reproductive physiology and feeding behaviour on the same host are the major factors helping in the differential diagnosis of *C. singularis* and *C. salviniae*.

3. Host-selection and speciation in aphids

Many aphids intimately associate themselves with specific host plants and evolve specific feeding, gall-making and reproductive strategies. Such a selection of host

plants with intrinsic modifications to feeding and egg-laying mechanisms has been used by taxonomists as the basis for distinguishing species in the super-family with close affinities to the plant groups they are associated with (David 1977). For instance, there are some species which, due to geographical isolation, have secured new hosts in south India. *Acyrtosiphon gossipii* Mordwilko, usually feeds on some plants of Malvaceae and Leguminosae mainly in dry areas in north-east Africa and West Asia (Eastop 1971). In south India, it is exclusively found on a totally different plant, *Sesbania grandiflora*, in wetlands. It has not been possible to transfer it to other plants like *S. speciosa*, *Medicago sativa* or *Vigna catjang*. Though *Sesbaniae* David was described as a distinct species, Eastop (1971) feels it could be treated as a subspecies on account of its shorter cauda compared to those on Malvaceae.

4. Pollination biology and speciation in plants

The richness and diversity of orchid speciation depends upon both the variety of pollinators to which they have become adapted and the various ways in which pollination is effected. Among the more interesting vectors that orchids have added to their repertory are males belonging to various species of Hymenoptera. An example now familiar is the phenomenon of pseudocopulation, according to which the flowers attract sexually active males by mimicking females of a particular insect species with respect to the overall form and colour pattern of the lip, and more particularly by subtle odors, produced by specific sesquiterpene lactones and hydrocarbons, as well as coatings of hairs on the lip, which produce a tactile stimulus for their copulation reaction. As was clearly demonstrated by Williams and Dodson (1972), male bees act as specific pollinators by gathering fragrant compounds, transporting them to courtship area and using them as sex attractants, the orchid flower serving as a sort of 'beauty parlor' for the sexually ambitious male.

The effectiveness of these pollination mechanisms in producing the morphological diversity by which botanists recognize and separate species was shown by Grant (1949) in a survey of the diagnostic characters used by botanists for separating species in a great number of different genera. In genera of plants adapted to pollination by specialized animal vectors, a high proportion of the morphological characters upon which species distinctions are based are concerned with the perianth, stamens and stigmas, which constitute the pollination mechanism. On the other hand, diagnostic characters in groups pollinated by less specialized vectors, or by wind or water, are based upon characters associated with vegetative adaptations or with seed development and dispersal.

5. Species problem and speciation

According to Mayr (1940, 1963), 'species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups'. Dobzhansky (1970) states that 'species are systems of populations; the gene exchange between these systems is limited or prevented by a reproductive isolating mechanism or perhaps by a combination of several such mechanisms'. Both these authors stress that *absolute* reproductive isolation cannot be used as the criterion of

specific distinctness. Furthermore, every species is at the same time a reproductive community, a gene pool, and a genetic system (White 1978). Speciation is, simply the evolution of a new species. It is the evolution of a new discrete morphological array of forms that interbreed only among themselves. Sibling species are reproductively isolated but are almost identical in outward appearance. Populations that are neither good races nor good species but are connected by a reduced amount of interbreeding and gene flow are recognised as 'semispecies' (Grant 1971).

Speciation is one of the main ways by which living organisms adapt in order to exploit the diversity of environments available to them. Three essential models of speciation are traditionally distinguished. In allopatric model, a single ancestral species population becomes separated into two by a geographical barrier that individuals cannot cross. However, Mayr (1978) feels that it would seem advisable to define allopatric in terms of dispersal barriers rather than purely geographically. The next stage is for the two populations to undergo different evolutionary changes in their different environments. If they diverge enough, the two populations may be classified as different geographical races, and then if they are unable to interbreed, they become different species. According to the sympatric model, much the same process of divergence can take place without the geographical separation of the populations but through instantaneous appearance of reproductive isolation. A third model of speciation, intermediate between the two extremes, is called parapatric (or semi-geographic) speciation. In this form, the divergence takes place between contiguous, rather than separated or overlapping populations. Many speciation events have been split into allopatric and sympatric categories when in fact they may have represented a homogeneous category from a population-genetic perspective. For these reasons, Templeton (1981) has given a population-genetic classification of the modes of speciation (table 1). There are two basic categories: divergence and transilience. Under divergence, the isolating barriers evolve in a continuous (but not necessarily slow) fashion, with some form of natural selection, either directly or indirectly, being the driving force leading to reproductive isolation. Transilient modes involve a discontinuity in which some sort of selective barrier is

Table 1. Modes of speciation (after Templeton 1981).

Type of speciation	Basic mechanism
Divergence	
Adaptive	Erection of extrinsic isolating barrier followed by independent microevolution
Clinal	Selection on a cline with isolation by distance
Habitat	Selection over multiple habitats with no isolation by distance
Transilience	
Genetic	Founder event causing rapid shift in previously stable genetic system
Chromosomal	Inbreeding and drift causing fixation of strongly underdominant chromosomal mutation
Hybrid maintenance	Hybridization of incompatible parental species followed by selection for maintenance of hybrid state
Hybrid recombination	Hybridization of incompatible parental species followed by inbreeding and selection for stabilized recombinant

come by other evolutionary forces. In a nutshell, divergence occurs because of isolation, transience in spite of selection (Templeton 1981).

Sympatric speciation in phytophagous insects

Extensive work on phytophagous insects suggest that reproductive isolation can occur when two populations may evolve while both are inhabiting the same geographic area. It is quite common to find two or more 'biological races' of a given species in the same locality, differing little or not at all in appearance, but nevertheless quite distinct in their food preferences. It has been stressed by Janzen (1968) that species of host plants of phytophagous insects may be viewed as islands in their relationship to the insects that feed upon them and that colonization of a new host is analogous to immigration to an unoccupied island.

Subspecific differentiation in Hyponomeuta padella

Caterpillars of the moth *H. padella* feed on apple and on hawthorn trees. There is considerable variation in the colour of the fore wings of the adults, from dark grey to white, but although the dark forms are commoner among moths reared from caterpillars from hawthorn, and white forms commoner in those reared from caterpillars from apple, the differences are only one of the relative frequencies of the different forms on the two varieties, and the adults are otherwise indistinguishable in appearance. However, the adult moths can usually be distinguished by their egg-laying preferences, since about 80% of individuals reared on apple lay their eggs on apple and about 90% of those from hawthorn lay their eggs on hawthorn. There is a strong tendency for moths to mate with partners raised on the same food plant; in experimental conditions such assortive matings were about twice as common as random matings between individuals raised on different plants. Finally, the caterpillars show a strong preference for the food plant on which their mothers were raised, although they can be induced by starvation to feed on the wrong food plant; in such cases the resulting adults are often infertile. In this case, then, the two races are almost completely isolated, since some interbreeding probably takes place in the wild; they are best regarded as subspecies of a single species.

Sympatric speciation in Rhagoletis

The most detailed, and hence the most significant studies on host races of insects have been carried out on the fruitflies of the family Tephritidae (Bush 1966, 1969a, b, 1975a, b). Bush has studied especially the genus *Rhagoletis* in north America. He has considered 3 species groups in detail. The *Suavis* group includes 5 species (from the eastern United States to Arizona and Mexico) that feed on a total of 8 species of nuts (*Juglans* spp.) without showing any particular specificity. They may well have speciated allopatrically. The *pomonella* group includes 4 sibling species, each of which infests fruits of a different plant family. Although difficult to separate on morphological grounds, these siblings seem to be completely isolated reproductively and are quite different biologically. *R. pomonella* originally infested only hawthorn (*Crataegus*) throughout the eastern United States, but a host race on introduced

apples appeared in the Hudson river valley about 1864 and rapidly spread until by 1916 it extended across the Great lakes region to the vicinity of Winnipeg. In the southern United States the species is still only represented by the hawthorn race.

At the present time there are minor differences in size, number of postorbital bristles, and ovipositor length between some sympatric populations of the two races. There is also striking differences in their seasonal cycles. Both have a single annual generation, but the emergence period of the apple race is from June 15 to the end of August (with a peak on July 25), i.e. about a month before the maturation of the apples, whereas the hawthorn race emerges between August 5 to October 15 (with a peak about September 12), i.e. approximately a month before the maturation of the hawthorn fruits. This allochronic isolation may prevent gene exchange between the two races.

Another allochronically isolated race of *pomonella* infests native and cultivated plums in the eastern United States. It emerges considerably earlier than the apple race, in accordance with the earlier maturation of the plums. And in the 1960s a cherry race made its appearance for the first time in Wisconsin. The cherry race must have arisen from the apple race, since the gap between the time of maturation of cherries and haws is too great for a direct shift to have taken place.

Thus, host races that are effectively isolated genetically can arise in this species from time to time when new host plants become available. A most important fact is that the insects are attracted to their host plants by visual and olfactory cues and that mating takes place on the fruits being then followed by oviposition. Bush (1974) points out that the biology of oligophagous and monophagous tephritids makes an allopatric origin of new host races highly unlikely. Migration of a few individuals to an area where a new species of fruit was present would have no effect unless these had the right genetic constitution for recognising, ovipositing and surviving on it. Most dispersal in *Rhagoletis* takes place before mating, so that both males and females would have to be attracted to the same fruits.

In place of such improbably allopatric models, Bush (1974) puts forward the following postulates in a sympatric model of *Rhagoletis* speciation (after White 1978):

- (i) The old and new host plants must occur in the same area.
- (ii) The maturation times of the two host species must overlap.
- (iii) Diapause and emergence times must be under genetic control.
- (iv) Orientation to and selection of a host plant due to a chemical cue.
- (v) Host selection is due to one main genetic locus.
- (vi) h_1h_1 individuals are attracted to the original host; h_1h_2 individuals are attracted to both hosts, but because of modifiers (polygenes) will move preferentially to the original host; while h_2h_2 individuals are only attracted to the new host.
- (vii) Survival on the original and new host is controlled by another locus. s_1s_1 individuals survive on the original host; s_2s_2 individuals survive on the new one; s_1s_2 individuals survive on either.
- (viii) Since mating occurs on the host plant, a high degree of homogamy is expected.

Under these circumstances one would expect that disruptive selection would lead to the rapid evolution of two races, one homozygous for $h_1h_1s_1s_1$, the other for $h_2h_2s_2s_2$. If the time of maturation of the two kinds of fruits is different, disruptive selection would also be expected to occur for the genes controlling the time of

emergence. Thus Bush and other workers on host races in Tephritidae have built up a strong case for the view that a few changes at key loci but not a major genetic revolution i.e. the replacement of a large proportion of the alleles in the original genotype can indeed lead to a switch in host plant. However, it is clear that once two sympatric host races are in existence there will begin a long process of disruptive selection, involving a change in the seasonal cycle of the new race and changes in those body and wing patterns (and perhaps behaviour patterns as well) that serve as cues in the mating ritual.

The process of differentiation into sympatric host races is explained well by Smith (1975). If for any reason a female of, for example, a plant-eating species lays her eggs on an unusual plant, two things will follow. First, the larvae will be exposed to new conditions, and intense selection of genotypes adapted to those conditions is likely; high mortality of forms reared on a new host species confirms this. Second, the females which develop on the new plant will tend to lay eggs on that plant, not because they tend to differ genetically from the rest of the species, but because they have been conditioned during larval life. Further, in species which mate soon after emergence, there is a fair probability of a female which has developed on the new host plant mating with a male from the same plant or group of plants, simply because she is more likely to meet such a male. In this way a population of insects can arise which, although not completely isolated reproductively from the rest of the species, may yet be sufficiently isolated by its habits to diverge genetically from the rest of the population. At first the main genetic change to occur in such a population will be that resulting from the intense natural selection for the capacity of the larvae to survive on the new host plant. However, any genetic changes which reinforce either the tendency to lay eggs on the new plant, or to mate with members of the newly adapted population, will be favoured by selection.

There are relatively few groups of animals in which host races equivalent to those of Tephritidae are well-known and fully documented. Monophagy, however, is equally shown in such groups as the gall-making cecidomyids and Cynipids, the agromyzids (Nowakowski 1962), most groups of parasitic hymenopterans, some aphids and coccids and certain groups of lepidopterans and sawflies. In Coleoptera there are a number of phytophagous and wood-boring families in which the species are closely adapted to a single host plant or a small range of related species. All of these are groups in which the occurrence of sympatric speciation seems probable, on the same kind of basis as in the tephritids. Leafhoppers (Cicadellidae) are another group in which it is very likely that sympatric speciation is occurring (White 1978).

6.3 The role of host plants in the speciation of treehoppers

Enchenopa binotata is a polyphagous membracid that occurs from Panama throughout eastern north America. In north America, it has a single generation per year and is found on 7 species of host plant. These hosts (*Ptelea trifoliata*, *Cercis canadensis*, *Juglans nigra*, *J. cinerea*, *Viburnum prunifolium*, *Celastrus scandens* and *Robinia pseudoacacia*) are evolutionarily diverse and sympatric throughout the eastern United States. Wood and Guttman (1981) have shown that *E. binotata* in north America is a complex of reproductively isolated taxa which have diverged along host lines. *Enchenopa* on each host differs (Wood and Guttman 1983) in

(i) the colouration of nymphs, (ii) oviposition sites, (iii) nymphal feeding sites, (iv) seasonal and diurnal patterns of oviposition and (v) the number of eggs per egg mass.

When females are given a choice of host plants on which to oviposit, they select the host on which they were raised. When males and females from all hosts are confined to a single cage, there are few matings by insects of mixed host origin and the length of mixed matings are considerably shorter. Even under conditions imposed by a cage, mating tends to occur on the host on which females were raised. Assortive mating within host plant species is almost assured considering disparate life histories, the mating system and the insular nature of treehopper populations. Host plants or individual trees may function as evolutionary islands with their isolated populations of coevolving herbivores as pointed out by Janzen (1968), Opler (1974) and Edmunds and Alstad (1978).

Allochronic life histories are important in maintaining reproductive isolation among members of this complex. Eggs hatch on each host (with the exception of *C. canadensis*) about the time the host is in flower. Allochronic egg hatch combined with differences in maturation produces temporal difference in mating; differences in the time of day that mating occurs further reduce the possibility of hybridization. Allochronic and diurnal differences in mating reproductively isolate adults from 4 of the 6 hosts. Members of the last pair are effectively isolated from each other by allochronic flight activity which occurs about a week apart. On one host, almost all flight activity occurs before mating begins on that host; hence, there is very little flight by either sex once mating begins and virtually none after oviposition starts. Ovipositional attractants in egg froth tend to keep females on their hosts. In fact, movements by males and females throughout the summer, even among nearby conspecific hosts, is almost non-existent.

Electrophoretically, *Enchenopa* from each host differ in the frequency and fixation of electromorphs even when collected from 2 adjacent tree species. There were even electrophoretic differences in the *Enchenopa* among individual conspecific trees located very close to each other. Genetic distances calculated from electrophoretic data indicate that *Enchenopa* on *J. nigra* diverged first, then divergence on *P. trifoliata*, *R. pseudoacacia*, *C. canadensis*, *V. prunifolium* and *C. scandens* followed in the order given. Estimates of the time of divergence by means of the molecular time clock suggest that speciation has been recent i.e. within the last 250,000 years.

Wood (1980) postulated that north American *Enchenopa* may have diverged from a tropical polyphagous ancestor. This ancestral stock encountered selection pressures to coordinate its life history with the phenology of newly exploited hosts resulting in a shift from a multivoltine life history. Colonization of north American hosts with differing phenologies and of differing nutritional quality resulted in difference in maturation that prompted allochronic life histories. Further genetic differentiation was promoted by the relative temporal permanence and spatial heterogeneity of host resources which encouraged low vagility. In this proposed model, Wood and Guttman (1981) ultimately feel that slight life history shifts of *Enchenopa* in response to host plants combined with behavioural and ecological factors are all that are needed to produce reproductively isolated species.

7. Conclusion

Since complete geographic isolation, if continued long enough, will inevitably lead

to speciation, we must expect that even among groups whose ecology, vagility and population structure is most favourable to sympatric speciation, there will be some cases of strictly allopatric speciation. The converse, however, does not hold; there are almost certainly groups whose general mode of life makes it impossible for reproductive isolation to develop without prior geographic isolation (White 1978).

Finally, it cannot be gainsaid that a thorough understanding of the dynamics of insect-plant interactions will pave the way for unravelling the mysteries of systematics and speciation in phytophagous insects.

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Azadirachtin—A naturally occurring insect growth regulator

B SUBRAHMANYAM

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India

Abstract. The well known and most useful property of the neem tree is the antifeedant property to insects expressed even in crude extracts. Azadirachtin has the highest biological activity and in addition to antifeedant property, it also produces developmental abnormalities in almost all insect orders. The chemical structure of azadirachtin has been determined unequivocally and the radio labelling opened the possibility of biochemical, metabolic and autoradiographic studies. The timing and titre of the two morphogenetic hormones are altered leading to loss of coordination of developmental events. This effect on morphogenetic hormones could be traced as the influence of azadirachtin on the neuroendocrine system which secrete the tropic hormones that eventually control the activity of corpora allata and prothoracic glands. Recent studies have shown that turnover of the neurosecretory material is poor in azadirachtin-treated insects leading to derangement of the hormonally controlled development.

Keywords. Azadirachtin; insect growth regulator; insect-plant relationship.

1. Introduction

The Indian neem tree, *Azadirachta indica* A Juss and its closely related China berry, *Melia azadirach* (Meliaceae) have been recognized since long for their unique properties. More than half a century ago, it was discovered that leaves of the neem tree contain chemicals strongly inhibiting feeding by the locusts that are polyphagous in nature (Chopra 1928; Volkonsky 1937). A plethora of studies have been made on the pesticidal properties, especially after the demonstration of strong antifeedant properties of the crude extracts of the neem seed kernels to locusts by Pradhan *et al* in 1962 and 1963 (for reviews see Gill 1972; Warthern 1979; Schmütterer 1981). A host of tetranortriterpenoids have been isolated from various parts of the neem tree and although all have not been tested for pesticidal properties, 3 compounds obtained from seeds are found to be active as feeding deterrents, toxicants and/or disruptants of growth and development against a variety of insect species and nematodes (Warthern 1979; Jacobson 1986). These compounds are meliantriol (Levie *et al* 1967), salannin (Henderson *et al* 1964) and azadirachtin (Butterworth and Morgan 1968, 1971). Azadirachtin has attracted worldwide attention not only as the most popular deterrent to insects but also as a promising growth regulator. The growing interest on the neem tree and azadirachtin in particular has not only led to detailed physiological and biochemical investigations on the action of this compound and development of pesticide formulations based on azadirachtin and neem oil but also to 3 international conferences exclusively on the neem tree. This review attempts to describe the biological effects of azadirachtin with special emphasis on its growth-disruptive action on insects.

2. Neem as the source of azadirachtin

The complicated structure of the molecule and the difficulties involved in the

synthesis, leave the natural material as the only source of azadirachtin at present. Besides the 3 important tetranortriterpenoids already mentioned, other compounds isolated from the neem seed are β -sitosterol, fatty acids, flavanoids and pentanortriterpenoids (Jacobson 1986). Several investigators have estimated azadirachtin content of the neem seeds from various sources, mainly from Asia and Africa. The yield varies markedly, depending on the origin of the seed material, ranging from 0.2% of commercial Indian seed to 3.5% of seeds from Ghana (Morgan 1981). Using thin layer chromatography of ethanol extracts, Ermel *et al* (1984) found that the best yields were from seeds obtained from Togo (6.2%) and India (3.5%). Further determinations from a number of samples by high performance liquid chromatography technique (Ermel *et al* 1987) revealed that samples from Nicaragua and Indonesia have the highest content (average 4.8%) and that samples from Togo, India, Burma and Mauritius have 3.3–3.9% of azadirachtin. Besides the large variation among individual trees, factors like light, temperature and humidity influence the azadirachtin content of seed kernels and exposure to UV radiation leads to significant loss (65%) within 14 h (Ermel *et al* 1987).

3. Azadirachtins

From the 1930s onwards, crude extracts from various parts of the neem tree were studied for insecticidal properties but it was not until 1968 the first biologically active component was isolated in pure form. Butterworth and Morgan (1968) obtained the first samples of azadirachtin. The large number of functional groups and the sensitivity of azadirachtin to acids and bases posed problems of structural analysis, although initial studies by Butterworth *et al* (1972) revealed the key molecular fragments. A complete structural assignment was made by Zannao *et al* (1975) but certain doubts on the structure remained which were not consistent with nuclear magnetic resonance (NMR) data. However, an unequivocal determination of the structure was achieved by Broughton *et al* (1986) from a crystalline (detiglyolated dihydroderivative) of azadirachtin that was suitable for X-ray diffraction studies. On the basis of NMR data Kraus *et al* (1985) also proposed the structure. The most critical structural element is the epoxy group at position 13–14. Removal of this group ends up with inactive compounds. Pure azadirachtin is a clear, white, microcrystalline solid (mp 149°C). It has been demonstrated that several azadirachtins occur in neem seed, having the basic triterpenoid structure as common to all of them (Rembold *et al* 1984; Forster 1988). In the seed cake 4 major components (A–D) were found in a proportion of 1 azadirachtin D:100 azadirachtin A:50 azadirachtin B:1 azadirachtin C. The structure of azadirachtin B has also been established recently (Rembold *et al* 1987b). Figure 1 shows the structure of azadirachtins A and B. Azadirachtins A and B differ in the position of tiglic acid, which is at C-1 for A and at C-3 for B. Another important difference in ring A substitution is the free OH-group of azadirachtin B in position 1. Azadirachtin A has been hydrogenated to dihydroazadirachtin A and also to its corresponding tritium labelled [22, 23– $^3\text{H}_2$] dihydroazadirachtin A (Rembold *et al* 1984) opening up the possibility of studies on the biochemical mode of action.

4. Biological activity in insects

The neem tree is well known for its insect-repellent and antifeedant properties.

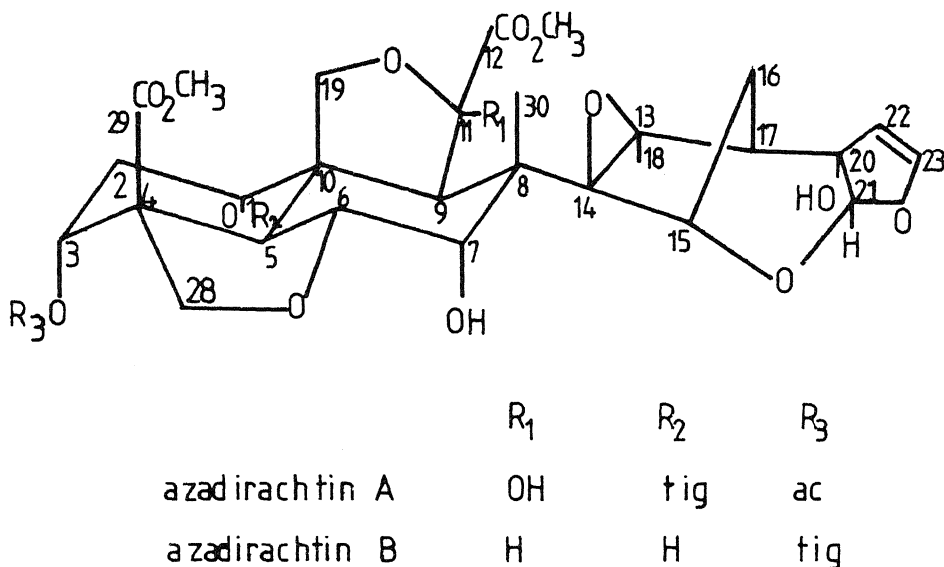


Figure 1. Structure of azadirachtins A and B (Rembold *et al* 1987a).

Centuries before commercial insecticides were available, farmers in the Indian subcontinent used neem derivatives to protect agricultural crops from insect attack. Of recent knowledge is the discovery of the insect growth regulating and sterilizing property of some pure fractions. In the light of the knowledge on the biological activity of crude material and the advances made after the isolation of the biologically active components, it would be worthwhile to examine the biological effects of crude material also.

4.1 Studies with crude extract and preparations

When a water suspension of neem kernels as low as 0.05% was sprayed on different crops and offered to the desert locust, the pest preferred to starve to death rather than feed on the treated leaves (Pradhan *et al* 1962). The laboratory findings were confirmed under field conditions demonstrating the most potent antifeedant activity. Such studies were later extended to several insect species. For a comprehensive account of the pesticidal properties of neem, see reviews by Warthern (1979) and Jain (1983). A total of 123 species of insects, belonging to diverse insect orders viz., Coleoptera, Diptera, Heteroptera, Isoptera, Lepidoptera and Orthoptera besides 3 species of mites and 5 species of nematodes are known to be adversely affected by neem preparations (Jacobson 1986). The growth-regulating effects were identified in crude methanolic extracts of neem leaves on east African coffee bug, *Antestiopsis orbitalis bechuana* by Leuschner (1972) though effects on fecundity could not be demonstrated. Later studies by Steets and Schmutterer (1975) and Schultz (1981) on *Epilachna varivestis* clearly demonstrated the growth disruption and sterilizing effects of neem components.

Studies with crude extracts on diverse species of insects were made extensively in the Federal Republic of Germany, USA and India, with a view to use them under field conditions. Some of the observations on growth and development are outlined.

Ascher and Gsell (1981) reported that the methanolic extract of neem seed (20–30 mg/kg) disturbed metamorphosis of *E. varivestis*. Effects on metamorphosis of *E. varivestis* grubs were enhanced 6-fold by the addition of a synergist 'Tropital' to the methanolic extracts (Lange and Schmutterer 1982). Application of the inexpensive 'enriched neem seed extract' at the rate of 6–12 mg/kg resulted in 100% mortality of the larvae of *Plutella xylostella*, *Pieris brassicae* and *Leptinotarsa decimlineata* (Schmutterer 1984). Topical application of crude extracts of neem seed on *Dysdercus fasciatus* resulted in mortality/asymmetry of body after moulting/reduced fecundity of the emerging adults (Occhse 1982). Topical application of acetone extracts of neem seeds and leaves on *D. cingulatus* led to supernumerary moults. Addition of methanolic seed extracts to artificial larval rearing medium of Medfly, *Ceratitis capitata* led to prolongation of instar, low food intake, low rate of pupation and hatching finally to reduced adult population to 16% of control (Steffens and Schmutterer 1982). However, Schauer (1984) showed that aphids sprayed directly with neem extracts were not appreciably affected. Laboratory and greenhouse tests have shown that after consumption of leaves, treated with enriched and formulated neem seed extract, the fecundity of *L. decimlineata* greatly reduced and some were completely sterile (Schmutterer 1987). The aqueous extract had strong antifeedant activity than the formulated solvent extracts. Aqueous extracts and formulated extracts of an azeotropic solvent mixture and of butyl methyl ether were evaluated against mosquito larvae by Zebitz (1987). These extracts showed toxic, growth-regulating and sterilizing effects on *Anopheles*, *Culex* and *Aedes* sp depending on the dose.

A 3–5% extract of neem kernel and neem oil showed antifeedant property against *Heliothis armigera* under field condition and 1–5% extract has strong antifeedant reaction against *Spodoptera litura*, on tobacco nurseries (Joshi *et al* 1978). Neem oil extractive, although had very poor antifeedant activity than crude extracts of neem cake against locusts and *S. litura*, was an effective mosquito larvicide (Attri and Ravi Prasad 1980). Feeding of sorghum grain and stem treated with neem kernel suspension to *H. armigera* and *Chilo partellus* led to developmental deformities (Jotwani and Srivastava 1984). Parmar (1987) has listed the biological effects of various neem extracts and neem oil on several Indian insects. These include synergistic, ovicidal and ovipositional deterrent effects besides the growth-disrupting effects.

Analysis of these studies suggests that the growth-regulating effects of the neem extracts are very well pronounced even when the crude extracts are applied. However, the variations in the efficacy are mainly due to variations in the quantities of the active principles and standardization of the product. Though neem is rich in several biologically active chemicals, the antifeedant and growth-regulating activities are primarily due to azadirachtin. The actual concentration of this compound in a crude preparation or an enriched extract and its loss due to degradation after application would ultimately determine the effect. These studies are of great value from an applied viewpoint. However, the growth-disrupting activity attributed to neem seed extracts or azadirachtin could, in some cases, be due to their antifeeding action, as antifeedants can also cause developmental deviations. Several physiological and biochemical studies have been made with pure azadirachtin. These are discussed in detail.

4.2 Antifeedant activity

Following the earlier demonstration of the antifeedant activity of neem extracts to the desert locust, the component chiefly responsible for this activity was identified as azadirachtin (Butterworth and Morgan 1968, 1971) and later its activity was demonstrated on diverse groups of insects, notably lepidopteran larvae such as the diamond back moth *P. xylostella*, cabbage butterfly *P. brassicae*, the tobacco bud worm *H. virescens*, the greater wax moth *Galleria mellonella* and the fall army worm *S. frugiperda* (Nakanishi 1975; Ruscoe 1972; Zanno *et al* 1975; Warthern *et al* 1978). The antifeedant activity on Lepidoptera can be best exemplified by the observation of Redfern *et al* (1981) on fall army worm that control larvae were 17 times larger (84.7 mg) than the test larvae fed on a diet containing 0.2 ppm azadirachtin (5 mg) and 85 times larger than the larvae fed on a diet containing 1 ppm. Larvae transferred to an untreated diet were unable to overcome the effects of treatment. Similarly, newly hatched house crickets *Acheta domesticus*, when fed on a diet containing 1–25 ppm, had less weight and development than controls and the effect was proportional to the concentration of the antifeedant (Warthern and Uebel 1981). Contrary to the observation on *S. frugiperda*, the nymphs of the house cricket that were fed on treated diet for 6 weeks, when transferred to normal diet, developed normally.

Antifeedant activity of azadirachtin is not a universal phenomenon in insects and exceptions do occur. Azadirachtin is not a feeding inhibitor in case of *E. varivastis* and it had been amply demonstrated by Rembold *et al* (1980) that growth disruption could occur independent of feeding inhibition. Yet another novel example is the blood-sucking bug *Rhodnius prolixus* which exhibits a clear dose-dependent effect. The ED₅₀ dose required for feeding inhibition is as high as 25 µg/ml of blood whereas that for moult inhibition is only 4×10^{-4} µg/ml (Garcia and Rembold 1984; Garcia *et al* 1984).

4.3 Effect on food consumption and utilization

To what extent azadirachtin would influence feeding and food utilization if administered through other routes, like injection, thereby avoiding the oral antifeedant action, in insects like the locusts which prefer to starve instead of feeding on azadirachtin-treated food? It was demonstrated that in the dose range of 1–8 µg of azadirachtin/g body weight, its injection caused dose-dependent reduction in body weight of final instar nymphs of the desert locust *Schistocerca gregaria* but even the highest dose did not cause absolute feeding inhibition (Rao and Subrahmanyam 1986). Such treatments significantly reduced the rate of feeding, growth and utilization of food to body mass. Similarly, a physiological dose of 2.5 µg/g injected into female migratory locust, *Locusta migratoria* did not cause starvation, though food consumption was reduced without significant loss or addition to body weight for one week post-treatment (Subrahmanyam *et al* 1989).

Such studies were also conducted on lepidopteran larvae by feeding sub-antifeedant concentrations added to food. Fagoonee (1984) allowed the cabbage web worm larvae to feed for 24 h on leaf discs treated with low concentrations of azadirachtin and showed that on the first day consumption index, larval body-

weight gain and growth index decreased with increasing concentrations, whereas the digestibility and efficiency of consumption of food to body mass were highest at highest concentration. Feeding subsequently on untreated food led to a rapid decline in food utilization efficiencies. Similar trend was also reported in case of *H. virescens* by Barnby and Klocke (1987). The antifeedant effects of azadirachtin are partly due to sensory detection and avoidance by insects (Schoonhoven and Jermy 1977) and partly due to centres that control feeding (Sieber and Rembold 1983).

4.4 Growth-regulating activity

Apart from the antifeedant effect, azadirachtin causes disorders in metamorphosis. It was Ruscoe (1972) who first demonstrated such an effect. Later these effects were reproduced in many insects species of several orders—on *L. decemlineata* and *E. varivastis* (Coleoptera) by Steets (1976) and Steets and Schmutterer (1975), on *Ephistia kuehniella* (Lepidoptera) and *Apis mellifera* (Hymenoptera) by Rembold *et al* (1980), on *D. koenigii* by Koul (1984) and *Bombyx mori* (Lepidoptera) by Koul *et al* (1987), on *L. migratoria* (Orthoptera) by Rembold and Sieber (1981) and Sieber and Rembold (1983), on *R. prolixus* (Heteroptera) by Garcia and Rembold (1984) and Garcia *et al* (1986). Typical disorders due to azadirachtin may be summarized as:

- (i) Induction of moult inhibition and mortality in a dose-dependent manner.
- (ii) Remarkable prolongation of instar duration accompanied by death or moult disruption. Locust fifth instar injected with a dose of 2 $\mu\text{g/g}$ body weight may continue without adult moult for more than 60 days (normal intermoult period being 9 days), and *Rhodnius* bugs may survive beyond 5 months without moult.
- (iii) Treated larvae remain in pharate condition unable to shed their old cuticle successfully. Weak ecdysial movements that could last for several hours accompanied by incomplete shedding.
- (iv) Incomplete or depressed resorption of the exuvial fluid.
- (v) Incomplete sclerotization and pigmentation of the new cuticle.
- (vi) Unplasticization of wing lobes leading to either wingless adults (in bugs) or adults with crippled wings (in moths).
- (vii) Severe deformities in head and thoracic appendages of the pupae of Holometabola.
- (viii) Disruption of oogenesis when injected into young adults (eg., locusts) and inhibition of embryonic development when injected into adults at the end of vitellogenesis.

5. Physiological and biochemical studies

The developmental changes caused at moult and the inhibition of oogenesis due to azadirachtin treatment pose several questions regarding its mode of action. Many workers have proposed that it might be acting at the hormonal level. However, so far a complete answer could not be obtained despite studies in this direction. It has been adequately demonstrated that such growth-regulating effects are not a consequence of its antifeedant action since topical application and injection of even

a single dose of azadirachtin interferes with moulting programme of larvae and egg maturation of the adult insect (Rembold *et al* 1984).

5.1 Influence on endocrine regulation of moulting

Rembold and co-workers have pioneered the studies on the influence of azadirachtin on endocrine regulation of moulting, taking *L. migratoria* as the model. Sieber and Rembold (1983) demonstrated that the haemolymph ecdysteroid titre pattern is modified and the peak suppressed in 5th instar *L. migratoria* nymphs following a single injection of azadirachtin at the beginning of the instar. Such modification of ecdysteroid titre is closely correlated with morphogenetic effects. Similarly, Mordue *et al* (1986) showed that ecdysteroid levels can be drastically reduced, delayed and extended or not significantly affected by azadirachtin treatment of *L. migratoria* final instar nymphs depending upon the time of injection.

Koul *et al* (1987) observed two types of effects depending upon the time of injection of azadirachtin into fifth instar larvae of *B. mori*. When injected prior to release of prothoracicotropic hormone (PTTH) i.e. into 0–3-day-old larvae, defective pupae were formed, whereas injection into 4–6-day-old larvae led to complete failure of pupation. It was further demonstrated by tissue culture and ligation experiments that azadirachtin has no direct effect on PTTH and prothoracic gland secretion.

Studying the effects of azadirachtin on the Asiatic corn borer *Ostrinia furnacalis*, Min-Li and Shin-Foon (1987) demonstrated that the ecdysteroid titre of the treated fifth instar larvae did not raise above 20 mg/ml of haemolymph throughout the instar whereas that of the normal larvae reached a peak (326.5 ng/ml) on the 6th day after moult and dropped on the 7th day to 46 ng/ml.

Azadirachtin decreased the cold induced elevation of juvenile hormone titre in the body of chilled wax moth, *G. mellonella* final instar larvae but had no effect on the allatotrophic activity of the brain. The ecdysteroid peak was higher and delayed by 24 h compared with the first ecdysteroid peak in controls (Malczewska *et al* 1988).

Ecdysteroid titres were too low for induction of ecdysis in the azadirachtin A-treated nymphs of *R. prolixus* (Garcia *et al* 1986). Ecdysone given orally (5 µg/ml) and juvenile hormone analogue (70 mg/insect) counteracted the ecdysis inhibition induced by azadirachtin (Garcia and Rembold 1984). Permanent larvae of the milk weed bug, *Oncopeltus fasciatus*, induced by azadirachtin doses show neither ecdysis nor apolysis and have a delayed and distinctly lower ecdysteroid peak (Dorn *et al* 1986).

5.2 Influence on ovarian development

The sterilizing effect of azadirachtin noticed in several insect orders brings out the fact that azadirachtin has a profound influence on the maturation of oocytes. Ecdysone and other ecdysteroids are synthesized in the cells of follicular epithelium at the end of oocyte maturation. Practically all the ovarian ecdysteroids are finally contained in the newly laid eggs and control cuticulogenesis during early development of embryo. After a single injection of azadirachtin into mature female locusts, follicle growth is inhibited. The length of the terminal oocyte remains only

1–2 mm compared to 6 mm in untreated females. Rembold and Sieber (1981) analysed the ovarian ecdysteroid levels of *L. migratoria* by radioimmunoassay (RIA) and showed that the ovarian ecdysteroid level in control increased near the end of vitellogenesis and reached a maximum within hours. Whereas injection of azadirachtin into females at the end of vitellogenesis (10–13 days after emergence) resulted only in very small amounts of moulting hormone in the ovaries. Ovaries of such insects were smaller and weigh only half that of controls. The number of mature oocytes was less, probably due to resorption.

Vitellogenesis is a process by which the fat bodies of the maturing adult insects synthesize specific proteins that are incorporated into the oocytes leading to their maturation. Juvenile hormone (JH) plays a vital role in this process (gonadotropic activity) and hence Rembold *et al* (1987c) analysed the haemolymph vitellogenins, JH as well as ecdysone levels of azadirachtin injected female or *L. migratoria*. These studies show that—(i) Azadirachtin significantly affects vitellogenin synthesis. Vitellogenin was detectable in females from day 6 after emergence, whereas in treated females it was absent until day 10 and appeared from day 14 onwards. (ii) In the control, a JH peak on day 8 precedes another on day 11, when ecdysone also reaches its maximum. These insects oviposit during days 13–15 and oviposition is succeeded by another JH peak at the start of the second gonadotropic cycle. Injection of 3 µg azadirachtin/g on day 4 after emergence leads to a completely different hormonal titre curves. The JH peak is seen only on day 15 and the ecdysone peak is seen on day 16 followed by oviposition on day 18. These observations clearly demonstrate the delay and mis-timing of the appearance of morphogenetic hormones leading to derangement of the gonadotropic cycle.

Does azadirachtin inhibit any of the enzyme systems involved in the biosynthesis of ecdysone? It was shown (Smith and Mitchell 1988) that azadirachtin inhibits in a dose-dependent fashion, the cytochrome P-450 dependent ecdysone-20 monooxygenase activity (an enzyme that converts ecdysone to its active metabolite 20-hydroxyecdysone) in homogenates of *Drosophila melanogaster* larvae, *A. aegypti* adult female abdomens or body or midgut of *Manduca sexta*. The concentrations required for 50% inhibition range from 1×10^{-4} to 4×10^{-4} M which are however an order higher than those required for moult inhibition. Accordingly, azadirachtin may not act at the level of this enzyme system.

A direct dependence of follicle cell differentiation and egg maturation on high JH titre has been already demonstrated for *L. migratoria*. However, synthesis of ecdysone by differentiated follicle cells is independent of JH or corpora allata and seems to be stimulated by neurohormones. Blockade of corpus allatum activity by azadirachtin hence seems unlikely. That azadirachtin has no direct influence on prothoracic gland activity has been already shown (Koul *et al* 1987).

The effect of azadirachtin on these two hormones could therefore be interpreted as an interference with neuroendocrine system which controls the ecdysone and JH synthesis. The tropic hormones viz., PTTH and allatotrophic hormone (ATTH) secreted from brain are involved in such a regulation. Histological studies have been made to understand the neuroendocrine control mechanism in azadirachtin-treated insects (Rembold *et al* 1984; Rao and Subrahmanyam 1986; Subrahmanyam *et al* 1989). The nymphs of *L. migratoria* that fail to moult even 40 days after treatment as well as the female adults whose gonadotropic cycle was suppressed due to azadirachtin show remarkable accumulation of stainable neurosecretory product

in the corpus cardiacum, a gland that plays a vital role in the storage and release of neurohormones. Hence, this is considered as the first step in the mode of action of azadirachtin.

However, these studies do not give a complete answer to the precise mode of action unless the fate of azadirachtin in the insect body is studied in detail. With the availability of radioactive probe some advances have already been made.

5.3 Fate of azadirachtin in insect body

Using the tritium labelled (22, 23- $^3\text{H}_2$) dihydroazadirachtin A, having the same biological activity as azadirachtin A it was shown by Rembold *et al* (1988) that a constant quantity of 0.4–0.5 μg of dihydroazadirachtin A/g body weight was recovered unchanged 5 days after injection of any physiologically effective doses (1.5–3 $\mu\text{g/g}$) into the female *L. migratoria*. It was remarkable to observe that Malpighian tubules account for 74% of dihydroazadirachtin A retained in the whole body. The site of its metabolism is not yet known but it is not degraded significantly by fat bodies and Malpighian tubules. In the Malpighian tubules it was localized in the basal and inner regions (Rembold *et al* 1988; Garcia *et al* 1989). It is hence likely that azadirachtin acts in its unchanged form through high affinity binding to organ-specific membrane receptors.

5.4 Effect on tropic hormones

Further studies (Rembold *et al* 1989; Subrahmanyam and Rembold 1989) reveal that azadirachtin concentrates more in the corpus cardiacum than in the brains and that while it does not penetrate the blood brain barrier, completely covers the corpus cardiacum gland structure. This suggests a strong possibility that while azadirachtin does not interfere with the neurosecretory activity of brain directly, may act on the functioning of the corpus cardiacum. Keeping this observation and the observation on the accumulation of neurosecretion in this gland in view, the turnover of the radio labelled (^{35}S -cystein) neurosecretory material was studied (Subrahmanyam *et al* 1989). In fact, it was shown that the turnover of the neurosecretion in the corpus cardiacum of azadirachtin-injected *L. migratoria* was very poor, leading to accumulation of the stainable neurosecretory material over a course of time. Hence, azadirachtin may influence the release mechanism of tropic factors leading to disruption of hormonally controlled processes like moulting and vitellogenesis.

6. Conclusion

Azadirachtin is one of the most thoroughly studied natural substances having potential as a pesticide of the future. Physiological and biochemical studies have yielded encouraging data on the possible mode of action. However, direct proof for the initial biochemical step, in the sequence of endocrinological events, affected by this molecule is yet to be identified and the exact interaction understood. Forthcoming investigations with new probes can certainly yield valuable information in this direction. In practice crude preparations and enriched

formulations, at least in the tropical countries, continue to be used for pest control in view of the ease of preparation and safety.

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Vascularisation of the placenta in some bats

A GOPALAKRISHNA and N BADWAIK

Department of Zoology, Institute of Science, Nagpur 440 001, India

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Abstract. Vascularisation of the placenta of several species of bats representing 8 families is reported. Maternal vascularisation in all these bats is effected by a few large vessels which pass through the entire thickness of the placenta and give rise to numerous radial branches on reaching the foetal surface of the placenta. Maternal blood is returned through placental tubules which empty into large venous channels at the utero-placental junction. Foetal vascularisation is brought about by two allantoic arteries which capillarise over the placental tubules, and a large allantoic vein. In the exchange areas there is mostly crosscurrent circulation of the maternal and foetal bloods. Only in emballonurid bats is there an haematoma from which maternal blood is not drained back but is absorbed by cells of the trophoblast of chorionic villi.

Keywords. Placenta; vascularisation; bats.

1. Introduction

The placenta of eutherian mammals is an unique structure in which two genetically heterologous components, namely the maternal uterus and the foetal chorion come into contact with each other during the gestation period. Each component has its own blood supply. Nevertheless, metabolic exchange takes place between foetal and maternal bloods although they are always separated by an interhaemal membrane whose composition and thickness varies among different mammals. The structure of the interhaemal membrane in the placenta has been described in many bats, but details of blood supply to and drainage from the placenta have not been described so far in any species except *Miniopterus schreibersii fuliginosus* (Gopalakrishna and Badwaik 1986). The placenta of *Miniopterus* is so unique, being composed of several moieties—a primary, two secondary and two tertiary moieties (Chari and Gopalakrishna 1984)—and vascularisation is so much unlike that in any other bat, that the placenta of this species cannot be considered as typical of Chiroptera. Typically, the placenta of bats is discoid and labyrinthine and the vascular pattern is nearly same except for minor variations in species in which the placenta becomes secondarily bidiscoidal or in which a deep pit occurs in the centre of the placenta, or an accessory placental structure such as an haematoma is formed. The present report is based on the examination of the placenta and umbilical cord at full term of the following species: *Rousettus leschenaulti*, *Pteropus giganteus giganteus*; *Cynopterus sphinx* (all belonging to Pteropodidae), *Taphozous longimanus* (Emballonuridae), *Rhinopoma microphyllum kinneari* (Rhinopomatidae), *Megaderma lyra lyra* (Megadermatidae), *Rhinolophus rouxi*, *R. luctus* (both Rhinolophidae), *Hipposideros speoris*, *H. ater ater*, *H. fulvus*, *H. lankadiva*, *H. bicolor pallidus* (all Hipposideridae), *Mormoops megaphylla*, *Pteronotus davyi* (both Mormoopidae), *Chaerephon plicata*, *Tadarida aegyptiaca* (both Molossidae), *Scotophilus temmincki* and *Pipistrellus ceylonicus chrysothrix* (both Vespertilionidae).

2. Materials and methods

Several specimens carrying full term pregnancy of all the species mentioned above except the two mormoopid species were collected from their natural roosts and their placental discs were cut out and fixed in neutral formalin or Bouin's fluid and sectioned serially at 8–10 μm thickness following the usual procedure of dehydration by passing through graded ethanol, clearing in xylol and embedding in paraffin. The sections were stained with Harris' haematoxylin and counterstained with eosin. The allantois of each species was likewise processed. Two full term uteri of each of the mormoopid bats studied here were obtained from the embryological collection at the Cornell University, Ithaca, New York, USA. Fixed gravid uteri of these specimens had been preserved in 70% ethanol. The tissues of these specimens were processed as in the other cases. The blood vessels were traced on graph sheets by examining serial sections and reconstructed from the tracings.

3. Results

On the basis of morphology of the placenta two types of definitive placenta are noticed among the bats studied here, viz. (i) discoid placenta as in most species of secondarily bidiscoidal placenta as in rhinolophid and hipposiderid bats and (ii) placenta composed of two moieties, a mesometrially located haematoma and a laterally located placental disc, as in *T. longimanus*. The vascular pattern in the placenta of molossid bats is described separately because of its uniqueness.

3.1 Maternal vascularisation

3.1a Simple discoid and bidiscoidal placenta: This is present in pteropids *Rhinopoma*, *Megaderma*, mormoopids, vespertilionids and molossids. There is a common pattern of maternal vascularisation of the placenta in all these species. Six to eight large afferent vessels enter the placental disc from the myometrial margin of the placenta and pass through the thickness of the placental disc until they reach the foetal surface of the placenta. A few of these vessels give rise to one or two branches midway, but these branches also reach the foetal surface of the placenta. After reaching the foetal surface each afferent vessel gives rise to numerous branches which run radially towards the periphery of the placental disc. They give rise to smaller branches which also run towards the periphery of the placental disc on the foetal surface of the placenta. All these vessels may be referred to as radial vessels (figures 1, 2, 10). Along their course the radial vessels open by small ostia into numerous placental tubules, which lie in the form of parallel columns during early gestation (figures 2, 11–13) but which become interconnected to form a labyrinthine structure during advanced stages of gestation (figure 14). Maternal blood is returned to the uteroplacental junction through the placental tubules which drain the blood into large venous channels near the uteroplacental junction (figure 2, 15, 16). The afferent vessels have an endothelial lining for a part of their length, but this may or may not persist in the radial vessels or in the placental tubules depending on whether the placenta is endotheliochorial or haemochorial.

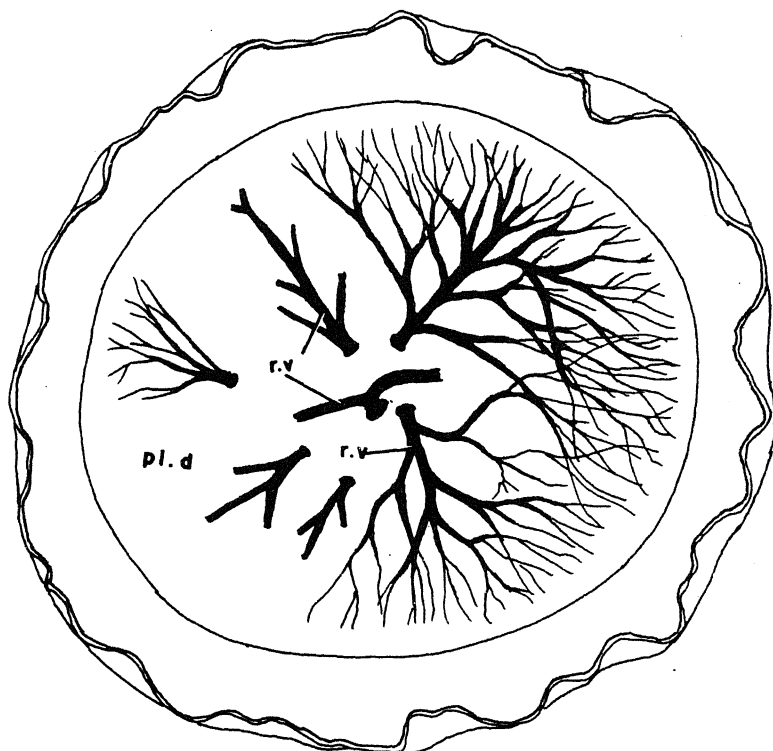


Figure 1. Radial vessels and their branches near the foetal border of a typical discoid placenta in a bat. pl.d, Placental disc; r.v, radial vessel.

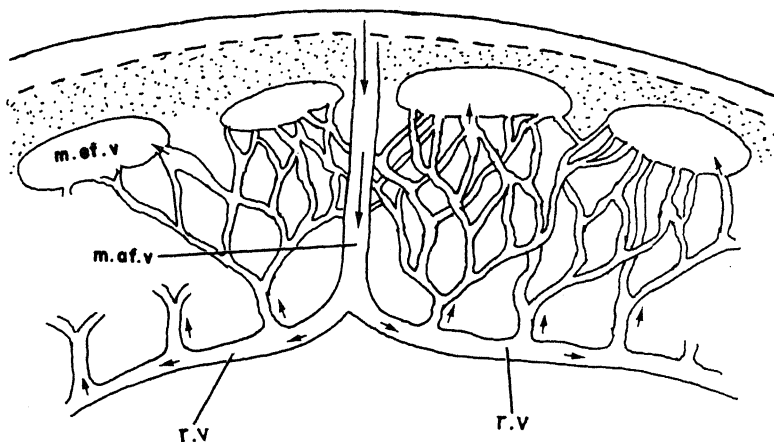


Figure 2. Section of a discoid placenta to illustrate maternal vascularisation of the placenta. m.af.v, Maternal afferent vessel; m.ef.v, maternal efferent vessel; other legends as in figure 1. The arrows indicate the direction of blood flow.

respectively. However, one or two layers of trophoblast invariably surround the radial vessels and the vascular channels in the placental tubules (figures 17–19).

In the haemochorial placenta (as in *Rousettus*, mormoopid and most

vespertilionid bats) the placental tubules are surrounded by an inner layer of syncytiotrophoblast and an outer layer of cytotrophoblast. In molossid bats, in which the placenta is haemomonochorial, only cytotrophoblast surrounds the placental tubules in the discoid placenta. Among the species having endotheliochorial placenta such as hipposiderid, rhinolophid and megadermatid bats and *Rhinopoma* the maternal endothelial cells in the placental tubules become hypertrophied and contain large, vesicular nuclei (figures 17, 19) until about the final quarter of pregnancy, when the endothelial cells become flat and possess fusiform, darkly staining nuclei.

In rhinolophid and hipposiderid bats the placenta is bidiscoidal or it has a deep and wide pit in the centre of the disc. Vascularisation of the placenta in these bats is similar to what has been described for the simple discoid placenta.

3.1b *Placenta with an accessory haematoma*: In *Taphozous* and all other emballonurid bats (Gopalakrishna 1958; Wimsatt and Gopalakrishna 1958; Sandhu 1986) the main placental disc lies on the lateral side of the gestation sac and the mesometrial part of the placenta becomes secondarily converted into an haematoma made up of numerous interconnected blood-filled chambers. The maternal vascularisation of the main discoid placenta is similar to that noticed in the normal discoid placenta (figure 3). However, the maternal blood supply to the

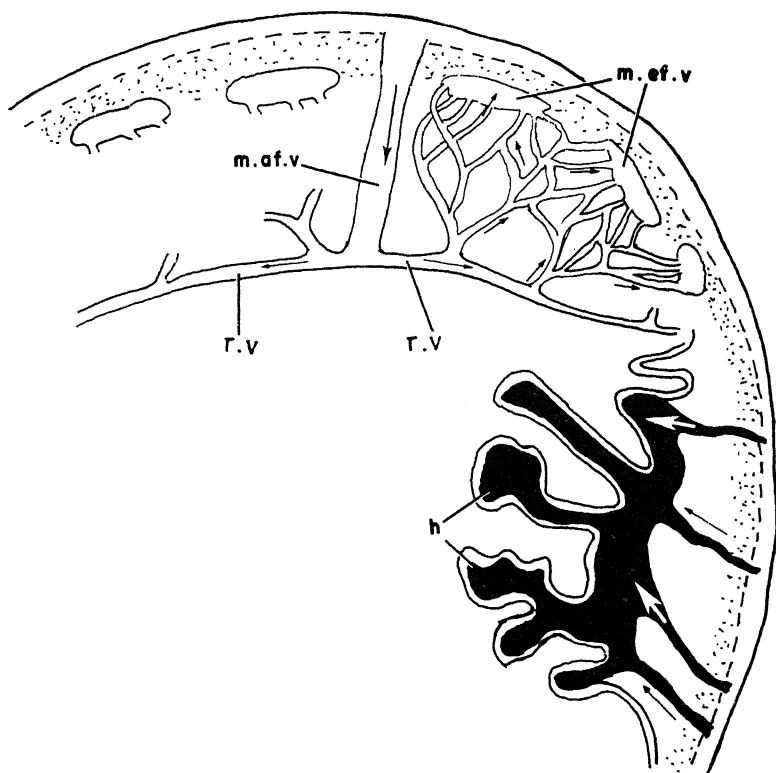


Figure 3. Maternal vascularisation of the placenta and haematoma in *T. longimanus*. h, Haematoma filled with maternal blood (solid dark areas). Other legends and arrows as in previous figures.

haematoma is different. A few small blood vessels coming from the myometrial side become ruptured, and blood is extravasated into 4–5 large broad interconnected chambers. Each chamber is divided into many smaller interconnected chambers by the formation of incomplete partitions composed of chorionic villi. Each chorionic villus is composed of a layer of large cubical to columnar cells, which are directly in contact with the extravasated maternal blood, and which form the maternal border of the villus. The central core of each villus contains allantoic mesenchyme composed of small fusiform or stellate cells and foetal blood capillaries. Maternal blood in the haematoma is absorbed by the trophoblastic cells of the chorion. Thus, there is no drainage of maternal blood from the haematoma.

3.2 Foetal vascularisation

Foetal vascularisation is brought about in all the bats through two allantoic arteries and one allantoic vein. The general pattern of foetal vascularisation is nearly same in the above mentioned two types of placenta with some minor variations in the cases where an accessory placental structure like an haematoma occurs as in emballonurid bats, or where there are two kinds of placenta as in the molossid bats. The allantois carrying the two arteries is inserted to the placental disc near about its centre in the simple discoid placenta (figure 4) and to the gap between the two discs in the bidiscoidal placenta. On reaching the placental disc the two allantoic arteries give rise to several branches which run along the foetal surface of the placenta towards the margin of the placental disc. Along their course

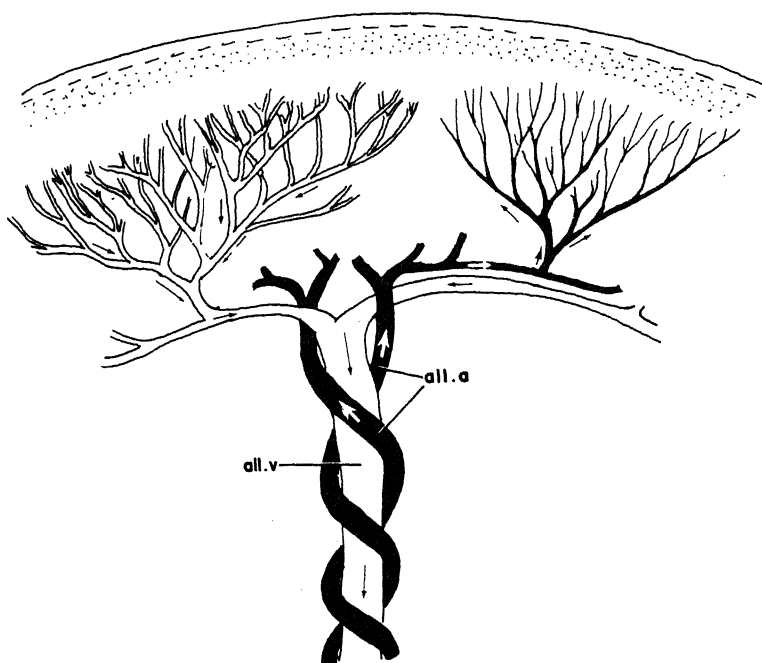


Figure 4. Foetal vascularisation of a discoid placenta. all.a, Allantoic artery; all.v, allantoic vein. Arrows indicate the direction of blood flow.

they give rise to smaller vessels which enter the placental labyrinth between the placental tubules. These vessels give rise to numerous arterioles which, in their turn, give rise to capillaries, which form plexes of capillaries surrounding the placental tubules (figure 5). Thus, the blood in the foetal capillaries flows nearly across and some times obliquely to the blood stream in the placental tubules. In some places the foetal capillaries may lie parallel to the placental tubules for some distance in which case the maternal and foetal bloods run in opposite directions. Complementary capillaries join to form venules which unite to form larger veins and finally the allantoic vein in the umbilical cord (figure 4). In the case of rhinolophid and hipposiderid bats, in which the placenta is bidiscoidal a vein returns blood from each placental disc and these two veins join to form the allantoic vein.

In the case of *Taphozous* a branch from one of the two allantoic arteries supplies blood to the haematoma (figure 6). After reaching the haematoma this vessel gives

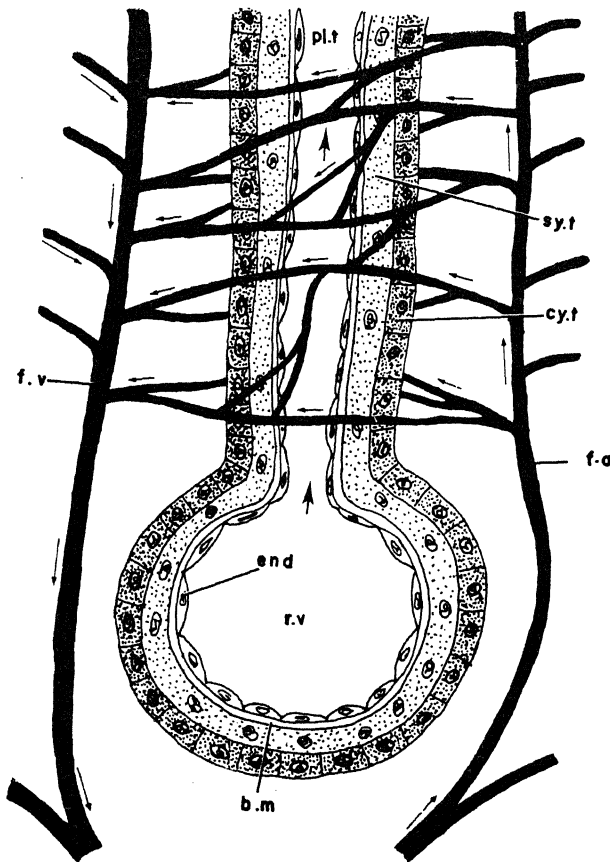


Figure 5. Arrangement of maternal and foetal blood channels near the foetal surface of an endotheliodichorial placenta. b.m, Basement membrane; cy.t, cytotrophoblast; end, endothelium; f.a, foetal arteriole; f.v, foetal venule; pl.t, placental tubule; sy.t, syncytiotrophoblast. Arrows indicate the direction of blood flow. Other legends as in previous figures.

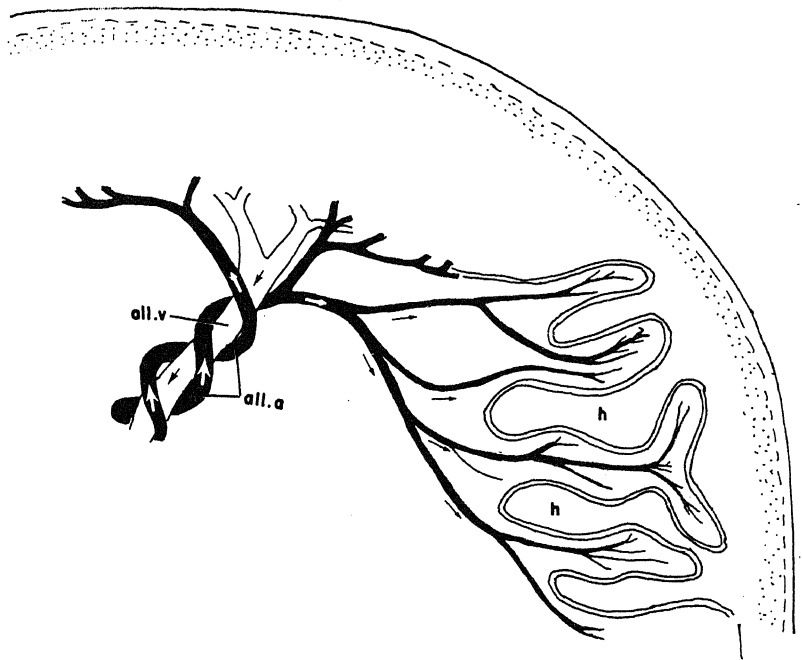


Figure 6. Foetal blood supply to the haematoma in *T. longimanus*. h, Haematoma. Other legends and arrows as in previous figures.

rise to numerous branches which carry blood to the chorionic villi in the haematoma. Complementary capillaries bringing blood from the chorionic villi form one vein which returns blood from the haematoma to a vein coming from the placental disc to form the allantoic vein.

3.3 Vascularisation in the placenta of molossid bats

The placentation in molossid bats is unlike that in other bats in several respects. Although, at full term the placenta in these bats occur as a thick bulbous discoid structure adjacent to the tubo-uterine junction on the mesometrial side of the uterus, a well developed diffuse placenta co-exists with the discoid placenta for a major part of the gestation period and disappears much after mid-pregnancy (Stephens 1962, 1969; Sandhu 1986; Gopalakrishna *et al* 1989). The diffuse placenta is endothelioidichorial and the discoid placenta is haemomonochorial. The vascularisation is different in the two kinds of placenta. Until a little after mid-pregnancy, the allantois spreads over the entire foetal surface of the placenta and two allantoic arteries give rise to several branches at the point where the allantoic stalk is attached to the placenta. Along their course these branches give rise to finer branches which spread on the foetal surface of the placenta. A few of these branches supply the discoid placenta, while the others give rise to smaller arterioles, which enter the placental labyrinth of the diffuse placenta where they give rise to capillaries. The vascular supply to the placenta is illustrated in figure 7. The capillaries form plexes which surround placental tubules in the diffuse placenta. A

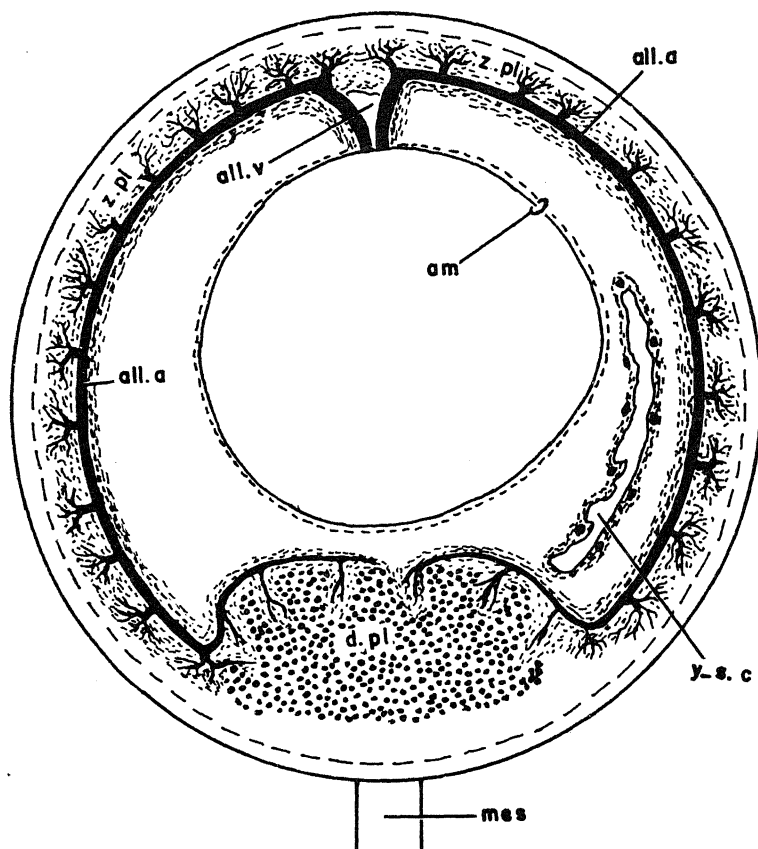


Figure 7. Foetal blood supply to the diffuse and discoid placenta a little after mid-pregnancy in *C. plicata*. Solid black lines indicate foetal vessels. all.a, allantoic artery; all.v, allantoic vesicle; am, amnion; d.pl, discoid placenta; mes, mesometrium; y-s.c, yolk-sac cavity; z.pl, diffuse placenta.

complementary set of vessels return blood from the diffuse placenta to the allantoic vein. With the progressive abolition of the diffuse placenta the two allantoic arteries vascularise only the thick discoid placenta on the mesometrial side.

During early stages of gestation the discoid placenta is in the form of a thick pad of cytotrophoblast. However, this becomes invaded by maternal vessels, which, during the final stages of gestation, occur as relatively large labyrinthine canals without endothelial lining but invested by one layer of cytotrophoblast cells. Foetal mesenchyme carrying foetal blood vessels invade the placental pad in the form of numerous thick projections, and these become interconnected resulting in compressing the maternal vascular channels invested by cytotrophoblast into placental tubules. Fine foetal capillaries lie mostly across the placental tubules, and most of them lie amidst adjacent cells of the cytotrophoblast surrounding the maternal vascular channels (figure 20). Under the light microscope the foetal capillary wall appears to be in contact with maternal blood in many places. The return of foetal blood from the placenta to the allantoic vein is similar to that noticed in the discoid placenta of other bats.

The thickness of the interhaemal membrane is highly variable depending not only on the number of layers of cells involved but also on the position of the foetal capillaries with respect to the placental tubules. It is naturally thicker in the endotheliochorial placenta than in the haemochorial placenta. Even among haemochorial placentae, in the case of mormoopid and vespertilionid bats (figure 18) the foetal vessels lie outside the layer or layers of trophoblast. However, in molossid bats studied here the foetal capillaries in exchange areas lie in the wall of the placental tubules between the cells of the cytotrophoblast with the result that the foetal capillary wall appears to be in contact with maternal blood suggesting almost an haemoendothelial condition. However, with the help of electron microscope Stephens (1969) demonstrated the presence of a very thin cytoplasmic membrane of the trophoblast separating the foetal blood capillary from the maternal blood in the New World molossid bat, *Tadarida brasiliensis cynocephala* (Stephens 1969; figure 27). This membrane cannot be defined by light microscope.

4. Discussion

From the foregoing it is evident that both maternal and foetal vascularisation of the

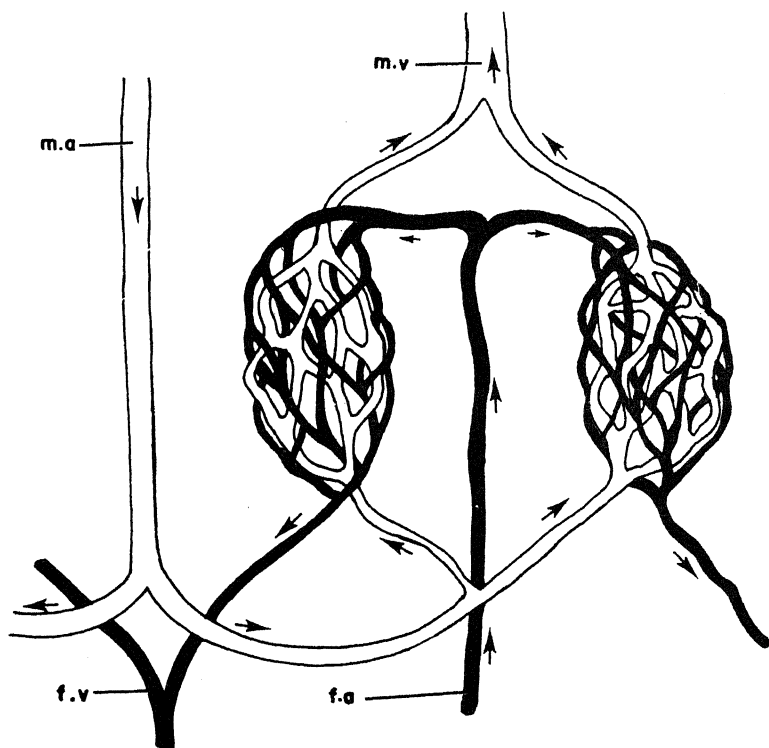


Figure 8. Drawing modified and adapted from Mossman's (1937) figure 6-e and (1987) figure 17.1-e to illustrate the countercurrent flow in the vascular pattern in an haemochorial labyrinthine placenta as that of the ground squirrel (*Citellus*). f.a, foetal artery; f.v, foetal vein; m.a, maternal artery; m.v, maternal vein. Arrows indicate the direction of blood flow. Mossman's drawing has been inverted here.

definitive placenta and its efficiency are nearly same in all the bats regardless of the finer structure of their placenta. Perhaps, only in the emballonurid bats the presence of an haematoma suggests that the discoid placenta of these bats may not be physiologically adequate with regard to the metabolic requirements of the foetus. This conclusion is borne out by the fact that foetal blood along with the haemocytes is directly ingested and metabolised by the chorionic cells and the products are transported to the foetus (Gopalakrishna 1958; Wimsatt and Gopalakrishna 1958). It is also evident that the efficiency of the discoid placenta as a remover of foetal metabolic waste is nearly same in all bats since the haematoma in the emballonurid bats does not, apparently, take any part in the elimination of foetal metabolic waste as there is no drainage of blood from the haematoma.

An interesting and important outcome of the present study is the light it throws on the possible manner of exchange of substances between foetal and maternal bloods in the placenta of bats. According to Mossman (1965, 1987) foetal and maternal capillaries lie parallel to each other and the flow of blood in the two is in opposite directions in labyrinthine placentae. He illustrated this concept in figures

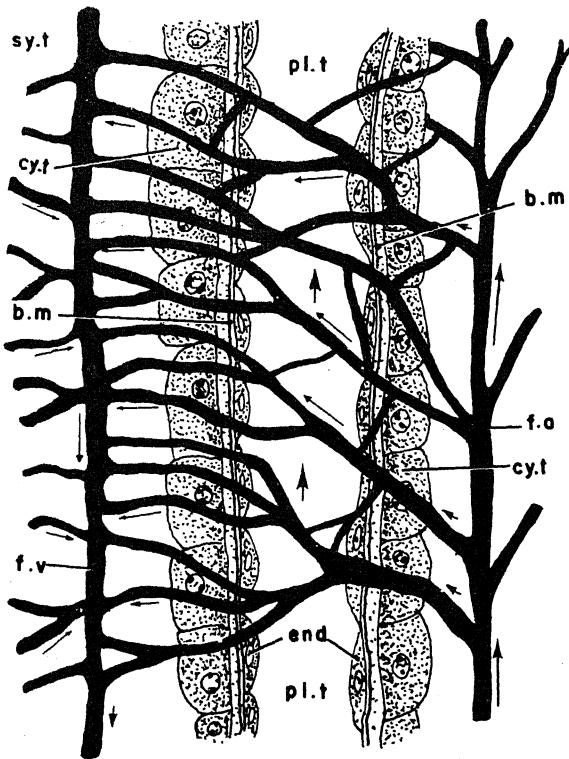
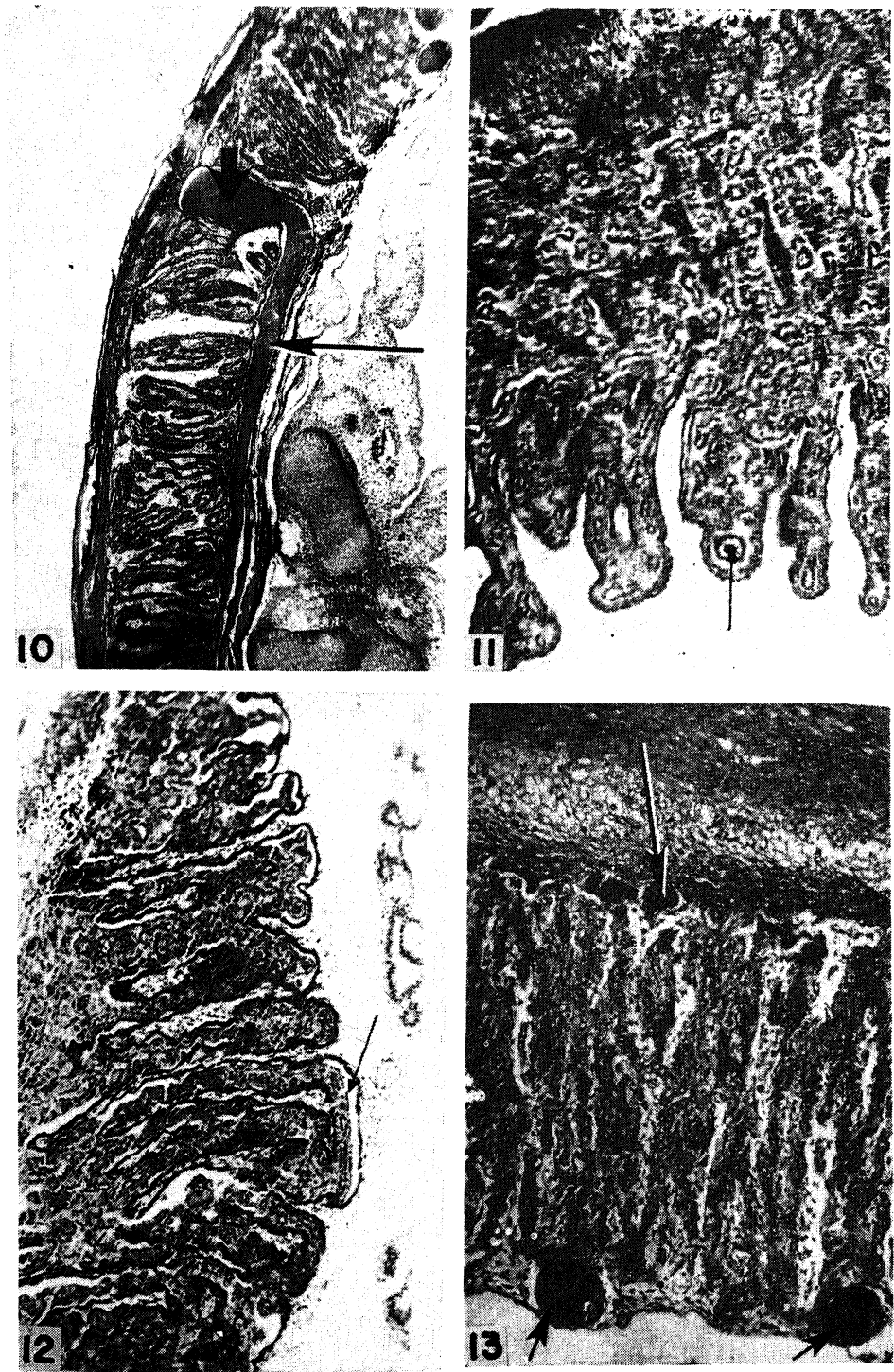
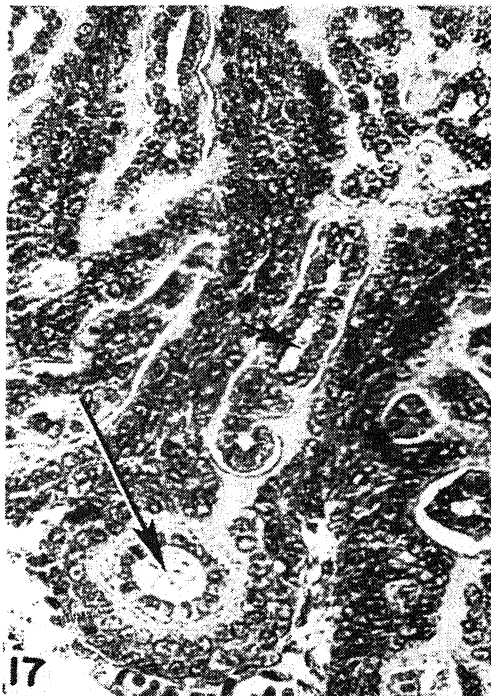
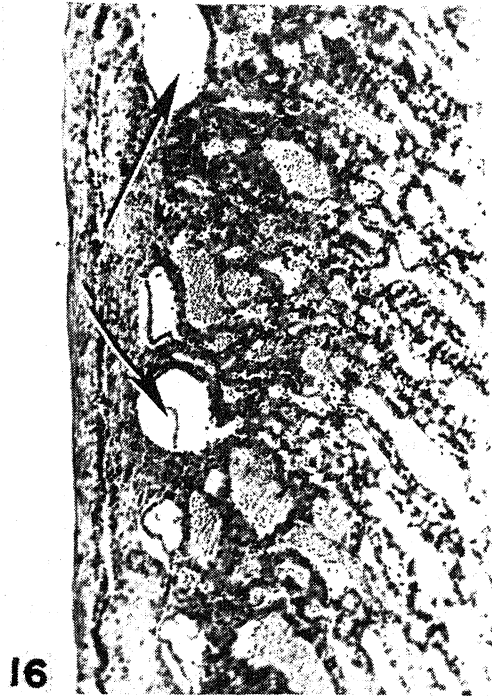
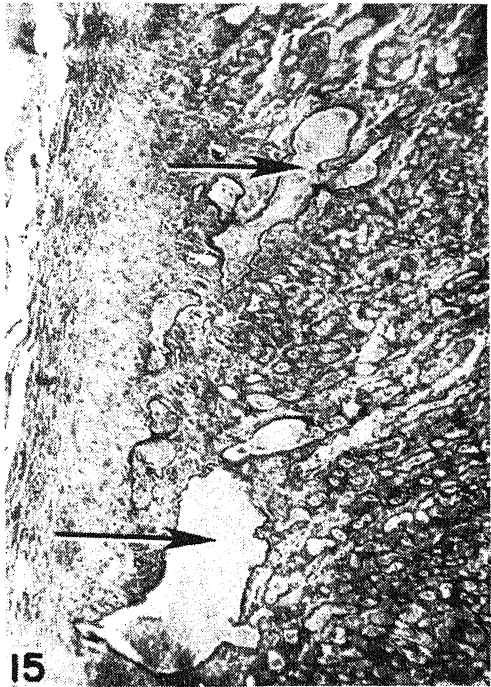


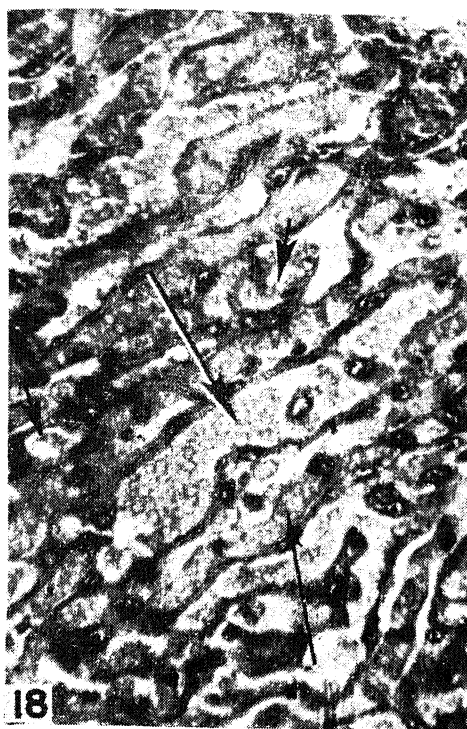
Figure 9. Reconstruction of the arrangement of foetal and maternal blood channels in about the middle of the thickness of the placental disc of the labyrinthine endothelioidichorial placenta of *M. l. lyra*. This scheme is equally applicable to the discoid labyrinthine placenta of other bats (except molossids) regardless of the nature of the interhaemal membrane. Foetal blood capillaries lie mostly across or obliquely to the placental tubules. b.m, Basement membrane; pl.t, placental tubule. Direction of blood flow is indicated by arrows.



Figures 10-13. For caption, see p. 303.



Figures 14-17. For caption, see p. 303.



Figures 18–21. For caption, see p. 303.

31.3 (1965) and 17.1 (1987). It must, however, be mentioned that he was referring to the haemochorial placenta of rodents. For easy reference this concept is illustrated with a slight modification in figure 8 in this study. As per this concept foetal arteries extend nearly up to the maternal margin of the chorionic villi, where they give rise to branches which turn back and give rise to capillaries, which lie parallel to the placental tubules or trophoblastic tubules (Mossman 1987). On anatomical grounds he suggested that there is a countercurrent exchange between foetal and maternal bloods in labyrinthine placentae. The present studies on the placenta of several species of bats belonging to diverse families has, however, revealed that foetal arteries, which enter the chorionic villi between the placental tubules and carry blood from the foetal surface of the placenta towards the myometrial border, give rise to branches soon after they enter the villi, and side branches from these form capillary plexes surrounding the placental tubules. Hence, these capillaries lie either across or obliquely, but rarely parallelly, to the length of the placental tubules (figures 18, 21). Therefore, the blood current in the foetal capillaries, which are intimately in contact with the placental tubules, is mostly across the blood current in the placental tubules (figure 9). Hence, there is a greater crosscurrent exchange than a countercurrent exchange between maternal and foetal bloods in the placenta. The schematic drawing given by Stephens (1969; figure 27) of the arrangement of foetal and maternal blood capillaries in the placenta of *T. b. cynocephala*, nearly describes the condition seen in the bats studied here except that the foetal capillaries lying parallel to the maternal vascular channels are less common in the bats studied here than shown to occur in *T. b. cynocephala*. The schematic drawing given by Stephens (1969) indicates that, while many foetal capillaries lie across the maternal blood channel, there is only one running parallel to the maternal blood channel. Yet, in the text the author stated, 'the predominant orientation of the foetal capillary bed is parallel to the maternal channel which they surround'. While describing the figure he stated, 'the main orientation of the foetal capillaries (F) round the maternal channel is longitudinal although numerous cross-over capillaries are present'. Further, in a highly complex 3-dimensional network of a biological system like the labyrinthine placenta, it is doubtful if the foetal blood capillaries form a network of parallel vessels to the network of placental tubules which themselves occur in a crooked and zigzag manner.

Acknowledgements

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Figures 10-13. 10. Section of the placental disc of *M. megaphylla*. Note the large maternal afferent blood vessels (thick short arrow) passing through the thickness of the placental disc and giving rise to a radial vessel (long arrow) running along the foetal margin of the placental disc ($\times 25$). 11. Part of the placenta of *M. l. lyra* to illustrate the radial vessel (arrow) and blood channels in placental tubules ($\times 80$). 12. Part of the section of the placental disc of *H. lankadiva* to show cut ends of radial vessels. In one place (arrow) a radial vessel has opened into 3 placental tubules ($\times 65$). 13. Part of the section of the placenta of *P. c. chrysothrix* to show the cut ends of radial vessels (short black arrows) near the foetal surface of the placenta, parallelly arranged placental tubules and maternal efferent vessels (shaded long arrow) at the utero-placental junction ($\times 110$).

Figures 14-17. 14. Part of the placenta of *H. lankadiva* to illustrate the labyrinthine nature of the placental tubules. Black short arrow points to the cut end of a radial vessel and long shaded arrow points to the maternal blood capillary inside the placental tubule ($\times 70$). 15. Part of the section of the placenta of *M. megaphylla* to illustrate the presence of large maternal efferent vessels (arrows) in the maternal margin of the placenta. Note several placental tubules opening into the efferent vessels ($\times 40$). 16. Utero-placental junction of *H. lankadiva*. Note the efferent maternal vessels (arrows) into which placental tubules open ($\times 65$). 17. Foetal margin of the placenta of *M. l. lyra* showing the detailed structure of a radial vessel (long shaded arrow) and placental tubule (short black arrow). The endothelial cells lining the maternal blood vessels within the placental tubules are hypertrophied and two layers of trophoblast envelop the maternal blood channel ($\times 300$).

Figures 18-21. 18. Part of the discoid placenta of *M. megaphylla*. Note the foetal vessels (short black arrows) lying outside the maternal blood channels (long shaded arrow). While some of the foetal vessels appear to lie parallelly to the maternal vessels (thin long arrow) most of them are only cut ends (short black arrows) indicating that they lie across the maternal blood spaces ($\times 350$). 19. Section of the placenta of *H. lankadiva* showing radial vessels (long shaded arrow) near the foetal surface of the placenta. Note the large foetal vessel (thick black short arrow) giving a branch between adjacent placental tubules ($\times 300$). 20. Part of the placenta of *C. plicata* to show the presence of cut ends of foetal capillaries (short black arrows) lying in the wall of maternal blood channels (long shaded arrow) between the cells of the cytotrophoblast ($\times 315$). 21. Section of the placenta of *H. lankadiva*. Note cut ends of foetal capillaries (arrows) and longitudinally running maternal blood vascular channel ($\times 350$).

Detection of chromosomal aberrations in the progenies of hydroxylammonium sulphate-induced recombinants in males of *Drosophila melanogaster*

G S MIGLANI, V P SINGH and K PREET

Department of Genetics, Punjab Agricultural University, Ludhiana 141 004, India

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Abstract. Chromosomal aberrations were detected in the progenies of hydroxylammonium sulphate-induced recombinants of *aristaless-dumpy* and *dumpy-black* regions of the males of *Drosophila melanogaster*. When males from homozygous lines derived from these male recombinants were crossed to females from an aberration-free stock, the chromosomal aberrations were not recovered in recombinant heterozygotes. Implications of these observations are discussed in relation to role of chromosomal aberrations in induced male recombination in *D. melanogaster*.

Keywords. Chromosomal aberrations; male recombination; *Drosophila*; hydroxylammonium sulphate.

1. Introduction

Sharma and Natarajan (1965) reported the occurrence of chromosome breaks in salivary chromosomes using 0.2% hydroxylamine and Parkash and Miglani (1978) observed 0.5% hydroxylammonium sulphate (HAS) to have caused specific chromosomal aberrations in *Drosophila melanogaster*. Induction of recombination in the regions *aristaless-dumpy* and *dumpy-black* of second chromosome of *D. melanogaster* with HAS was reported by Miglani *et al* (1990). One of the causes of non-reciprocal recombination in males of *D. melanogaster* was hypothesized to be breakage and reunion of chromosomes (Woodruff and Thompson 1977). It was thought worthwhile to test this hypothesis. The present investigation was, therefore, undertaken to explore the possibility of association of induction of recombination in *D. melanogaster* males with chromosomal rearrangements, using potent chromosome breaking agent, HAS.

2. Materials and methods

2.1 Stocks and probe

A wildtype laboratory stock, Oregon-K, and a mutant stock homozygous for 3 second-chromosome recessive markers, *aristaless* (*al*: 2-0-0), *dumpy* (*dp*: 2-13-0) and *black* (*b*: 2-48-5), were used. Gene symbols and map distances are as given by Lindsley and Zimm (1985). HAS with chemical formula $(\text{HO-NH}_3)_2\text{SO}_4$ and molecular weight 164.51, manufactured by British Drug House, England (Batch No. 334540), was used as a probe. HAS at 0.5% concentration, found to be most effective in inducing chromosomal aberrations in *D. melanogaster* by Parkash and Miglani (1978), was used in this study.

2.2 Treatment procedure and crosses

Treatment was given by rearing *Drosophila* larvae on food mixed with 0.5% HAS (9:1). Control experiments were also done simultaneously. Control and HAS-treated F_1 males were crossed with *al dp b* females, TC_1 progeny was screened for phenotype and sex.

2.3 Confirmation of male recombinants and study of TC_2 progenies

TC_1 male recombinants were again crossed with *al dp b* females genotypically/phenotypically and confirm the recovery of male recombinants. TC_2 larvae (3-5) were sacrificed for the study of salivary gland chromosomes for the presence of chromosomal rearrangements following the method of Singh (1989).

2.4 Construction of and studies on homozygous recombinant lines

From the TC_2 progenies of the TC_1 male recombinants recovered, males and females showing the same recombinant phenotype were inbred and appropriately recrossed until a line homozygous for a particular TC_1 male recombinant was constructed. Males from a particular recombinant line were crossed with females from an aberration-free stock, *al dp b* of *D. melanogaster*. Larvae (3-5) from each of the progenies were sampled randomly for examining salivary chromosomes for the presence of chromosomal aberrations, following the method of Singh (1989). All the experiments were done at $25 \pm 1^\circ\text{C}$.

3. Results

3.1 Induction and confirmation of recombinants

Number of TC_1 male and female parental and recombinant types observed, number of male recombinants further testcrossed and verified in *D. melanogaster* are given in table 1. Control (20) and HAS-treated (25) F_1 (+/*al dp b*) males yielded 27 (7 males+20 females) and 72 (20 males+52 females) recombinants, respectively. All the 7 and 20 TC_1 male recombinants recovered in control and HAS experiments, respectively, were confirmed genotypically/phenotypically in TC_2 generation. In TC_1 progenies of untreated and HAS-treated F_1 males, in *al-dp* region, 0.642 and 0.810, and in *dp-b* region, 0.357 and 0.902% recombination was, respectively, observed. Non-reciprocal recombination in both *al-dp* and *dp-b* regions was observed with HAS as the two complementary recombinant phenotypes in each of these regions did not appear in 1:1 ratio ($P < 0.05$). HAS-treated F_1 males exhibited non-Mendelian segregation, in pooled TC_1 progeny, at *al*, *dp* and *b* loci of *D. melanogaster*.

3.2 Examination of salivary chromosomes

As many as 4581 salivary gland cells were examined from 28 larvae of 7 TC_2

Table 1. Number of TC₁ male and female parental and recombinant types observed, number of male recombinants further testcrossed and verified in *D. melanogaster*.

	TC ₁ progeny	Number of TC ₁ male recombinants	
Phenotype	M + F	Further testcrossed	Verified
Number of F ₁ males testcrossed = 20			
Control			
+ + +	724 + 718		
<i>al dp b</i>	634 + 699		
+ <i>dp b</i>	5 + 8	5	5
<i>al</i> + +	0 + 4	0	0
+ + <i>b</i>	0 + 4	0	0
<i>al dp</i> +	1 + 4	1	1
+ <i>dp</i> +	1 + 0	1	1
<i>al</i> + <i>b</i>	0 + 0	0	0
	1365 + 1437	7	7
Total	2802	7	7
Number of F ₁ males testcrossed = 25			
HAS-treated			
+ + +	1013 + 1032		
<i>al dp b</i>	1093 + 1113		
+ <i>dp b</i>	12 + 10	12	12
<i>al</i> + +	0 + 11	0	0
+ + <i>b</i>	0 + 12	0	0
<i>al dp</i> +	8 + 17	8	8
+ <i>dp</i> +	0 + 1	0	0
<i>al</i> + <i>b</i>	0 + 1	0	0
	2126 + 2197	20	20
Total	4323	20	20

M, Males; F, females.

progenies of control TC₁ male recombinants whose phenotypes are given in table 1. Only one larva from TC₂ progeny of one control TC₁ male recombinant, + *dp* +, possessed one heterozygous inversion on the right arm of the second chromosome of *D. melanogaster* with breakage-union points 43A-44D. Out of 5171 salivary gland cells examined from 69 larvae of 20 TC₂ progenies of HAS-induced TC₁ male recombinants (table 1), 3 larvae sampled from TC₂ progenies of 3 different TC₁ male recombinants (all of phenotype + *dp b*), revealed 3 inversions, (i) 2L(26F-29F), (ii) 3R(84C-85CD) and (iii) 3R(82F-84C), and one deletion 3L(61A-62F). Inversion (i) and the deletion were detected in 2 different chromosome complements of one and the same larva.

We were able to construct homozygous recombinants only for one control and 7HAS-induced TC₁ male recombinants. When males from each TC₁ male recombinant homozygous line were crossed with females from aberration-free stock, *al dp b*, the resultant larvae possessed no such chromosomal aberration as could be detected under light microscope at 1,000× magnification.

4. Discussion

4.1 Non-reciprocal recombination

Non-reciprocal recombination was observed with HAS in *al-dp* and *dp-b* second-chromosome regions of *D. melanogaster*. As reviewed by Singh (1989), non-reciprocal recombination has been reported in *Aspergillus*, yeast, *Ustilago* and *Escherichia coli*. Singh (1989) also recorded that non-reciprocal male recombination was induced in *D. melanogaster* with bleomycin, dihydroxymethyl peroxide, ethyl methanesulphonate, formaldehyde, radiofrequency, ultraviolet light, X-rays and neutrons. Non-reciprocal recombination in males of *D. melanogaster* may arise due to induction of recombination, not by classical crossing over, as in females, but by chromosome breakage and reunion events as suggested by Woodruff and Thompson (1977). Alternatively, induction of recombination by classical crossing-over, as in females, but preferential elimination of one of the two complementary products may result in what we here observed as non-reciprocal recombination (Sharma and Swaminathan 1968).

4.2 Chromosomal aberrations and male recombination

Several workers have reported isolation of wild chromosomes having the ability to induce male recombination in *D. melanogaster* (Hiraizumi 1971; Kidwell and Kidwell 1975). A second-chromosome male recombination factor, MRF 31.1, induced inversions, duplications and deficiencies in both male and female germ cells, in addition to male recombination, in both second and third chromosome (Yannopoulos and Zacharopoulou 1980). HAS induced second and third chromosome rearrangements (detected in TC₂ progenies of some of the TC₁ male recombinants), in addition to male recombination (recovered in TC₁ generation) in second chromosome of *D. melanogaster*, in the present study. Although salivary cells were very carefully examined, the possibility that some of the chromosomal aberrations of small magnitude might have gone undetected is not ruled out. Therefore, it is not possible to work out a possible correlation between induction of chromosomal aberrations and male recombinations.

High frequencies of unstable chromosome rearrangements, including specific deletions and duplications were also induced by second chromosome MRF 23.5 (Yannopoulos *et al* 1982). Chromosomal aberrations, overlapping the *dp-b-cinnabar* region of the second chromosome of *D. melanogaster*, were detected in the TC₂ progenies of ethyl methanesulphate-induced TC₁ male recombinants of phenotype *black cinnabar* (Miglani and Mohindra 1985); these experiments were, however, not carried on further. None of the chromosomal rearrangements detected in the TC₂ progenies of HAS-induced TC₁ male recombinants of *D. melanogaster*,

in the present study, could be recovered when the larvae were sampled from recombinant heterozygotes obtained by crossing females from aberration-free stock (*al dp b*) with males from homozygous recombinant lines. This means that if any of these or other undetected chromosomal aberrations were directly or indirectly involved in induction of recombination in males of *D. melanogaster*, they were unstable and, perhaps, that is the reason they were not detected in recombinant heterozygotes. The involvement of chromosomal rearrangements in induction of male recombination in *D. melanogaster* is still an open question. If breakage and reunion of chromosome is actually involved in induction of non-reciprocal male recombination in *D. melanogaster*, as suggested by Woodruff and Thompson (1977), our contention is that such chromosomal rearrangements must be unstable or, if stable, of very small magnitude. Further experiments are in progress to understand the role of chromosome breaks/rearrangements in male recombination in *Drosophila*.

Acknowledgement

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Association of *Termitomyces* spp. with fungus growing termites

D K SIDDE GOWDA and D RAJAGOPAL

Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore
560 065, India

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Abstract. Among 5 species of *Termitomyces* spp. associated with *Odontotermes* spp., *Termitomyces microcarpus* was the most dominant on the mound surface of *Odontotermes redemanni* during the rainy season. This species was found to grow on the fungal comb fragments brought out by termites as the substrate for its growth. As a result, decrease in cellulose (5.9%), lignin (3.5%) nitrogen (0.54%), carbon (11.2%), C:N ratio (1.37), crude fat (0.48%), moisture (17.02%) and increase in ash content (20.15%) were observed. It was also observed that *Termitomyces microcarpus* was rich in protein (39.16–43.37%) and mineral content.

Keywords. *Odontotermes*; *Termitomyces*; mushrooms; fungus combs.

1. Introduction

Termites cultivate basidiomycete fungi *Termitomyces* in their nests. Certain species of *Termitomyces* are known to grow on the mound surface and also on other surrounding ground during the rainy season in the form of fruiting bodies as mushrooms. These mushrooms are highly priced for their delicacy and nutritive value as human food (Mukiibi 1973). Heim (1941) was the first to establish the genus *Termitomyces* from the fungal gardens of termites. Since then several species have been reported in symbiotic association with termites from different parts of the world (Alasoadura 1966; Oteino 1979; Purkayastha and Andrilla 1985). Literature on the chemical composition of fungal combs before and after growth of *Termitomyces* is scanty, except for the observations of Batra and Batra (1979) who reported low cellulose content on the newly emerged *Termitomyces* compared to the well developed combs/mushrooms. Therefore an attempt has been made to know the different species of *Termitomyces* associated with mound building termites, their chemical composition and nutritive value.

2. Materials and methods

Surveys were conducted during the rainy seasons of 1986 and 1987 at Bangalore, Tumkur and Chikkamagalur districts. Different species of *Termitomyces* mushrooms grown on the mounds of *Odontotermes* spp. and surrounding soil were observed. While collecting the different species of mushrooms, soil was excavated 2 cm away from mushrooms to find out its origin arising as the stipe from the fungal fragment. Freshly collected mushrooms were identified, using the keys given by Purkayastha and Andrilla (1985) and Natarajan (1975, 1979). Morphological descriptions of different species of mushrooms under field condition are given. During the same period several mounds of *Odontotermes redemanni* (Wasmann) were marked in the

grassland and on these the growth of *Termitomyces microcarpus* Berk and Broom was observed for a period of 3 days invariably after rains. Samples of fungal combs before and after growth of *Termitomyces* were collected and analysed for cellulose (Updegraff 1969), lignin (Mukhopadhyaya and Nandi 1979), ash, nitrogen (Jackson 1973) and crude fat (Allen *et al* 1974) contents. Similarly, mushrooms were analysed for nitrogen, mineral matter Ca, Mg (Jackson 1973) and crude fat (Allen *et al* 1974). Micronutrients such as Cu, Zn, Mn and Fe were determined using Spg atomic absorption spectrophotometer as outlined by Lindsay and Norvell (1978).

3. Results and discussion

The present investigation has revealed that 9 species of mushrooms were associated with 4 species of fungus growing termites viz. *Odontotermes obesus* (Rambur), *O. redemanni*, *O. wallonensis* (Wasmann) and *Macrotermes estherae* (Desneaux). The mushrooms belong to 5 genera of Agaricales (table 1).

The genus *Termitomyces* was closely associated with termites as its species were found growing on the mound and surrounding soil. Apart from this genus, *Cantharellus* sp. and *Collybia familia* were observed to grow in the galleries of *O. wallonensis* and on the surface of *O. redemanni* mounds respectively. Species of *Rusella* were also found to grow in the foraging areas of termite *M. estherae*.

More than one species of mushrooms were associated with certain termite species, as in the case of *O. redemanni* (5 species of mushrooms) followed by *O. wallonensis* (4 species). Based on the occurrence and distribution of mushrooms, *T. microcarpus* (86.11%) was most dominant followed by *T. clypeatus* (51.72%). *C. familia* and *Cantharellus* sp. were distributed on only 2.5% of the mounds observed. In the present study these species were reported for the first time along with their host association from this region. However, Natarajan (1975, 1979) and Purkayastha and Andrilla (1985) observed these species growing from an unspecified termite mounds. Likewise, Batra and Batra (1979) observed *T. albuminosus* and *T. microcarpus* associated with *O. obesus* and *O. gurudaspurensis* Holm. mounds respectively. Similarly, *T. microcarpus*, *T. indicus* and *T. badius* Oteino were found associated with *O. redemanni* and *T. clypeatus* and *T. indicus* with *O. wallonensis* in the present study (table 1).

Table 1. Association of mushroom species with fungus growing termites.

Mushroom species	Dominance (%)			
	<i>Odontotermes obesus</i>	<i>O. wallonensis</i>	<i>O. redemanni</i>	<i>Macrotermes estherae</i>
<i>T. albuminosa</i>	37.5	17.24	38.96	—
<i>T. clypeatus</i>	—	51.72	—	—
<i>T. microcarpus</i>	—	—	86.11	—
<i>T. indicus</i>	—	5.17	0.09	—
<i>T. badius</i>	—	—	3.17	—
<i>C. familia</i>	—	—	2.50	—
<i>Cantharellus</i> sp.	—	2.50	—	—
<i>Rusella</i> sp.	—	—	—	44.44
<i>Lepiota</i> sp.	—	—	—	11.11

The morphological characters of the mushrooms are given in table 2. *T. albuminosus* was the larger mushroom compared to other species. The mean length of the stipe (11.4 cm) and diameter of pileus (9.6 cm) were highest in the case of *T. albuminosus* and lowest in the case of *T. indicus* (3.26 cm) and *T. badius* (1.46 cm). The gills were adnexed to free in all the species observed.

3.1 Observations on the growth of *T. microcarpus*

Field observations during rainy season from July to October on the cultivation of mushrooms revealed that *T. microcarpus* was the common mushroom growing regularly on the mounds of *O. redemanni*. The mode of mushroom cultivation by the termites was almost similar in all the mounds observed. The reason for cultivating mushrooms by the termites themselves on the mound surface is not clearly understood. Generally, fresh fungal comb fragments along with the basidiocarps are harvested from the fungal combs inside the mound and deposited usually between the conical growth of the mound (Batra and Batra 1979; Natarajan 1975). After deposition, the mycelium strands grow through the comb fragments, fruiting bodies or the mushrooms begin to appear as very small knobs with enlargements on those strands, and they appeared to be button like structures in the beginning. They were enlarged and elongated, made their way towards the surface of the comb fragments. They appeared very small and grown from the size of pinhead to pea size. Further, they were divided into two main parts, viz. stipe and pileus. At this stage gills appeared on the under surface of the pileus. Later the cap grew in size (1.5 cm), the gills elongated and the stipe also increased in length (3.5 cm). About 90–135 mushrooms were recorded from each place, with 6 mushrooms per square inch. The total development took 36 h from deposition of comb fragments to full growth of the mushroom on the mound surface. These observations are in agreement with the findings of Atkinson (1961) in respect of the edible mushroom *Agaricus bisporus* from USA.

3.2 Chemical composition of fungal combs during growth of *T. microcarpus*

An analysis was made to determine the changes in cellulose, lignin, carbon,

Table 2. Morphology of *Termitomyces*.

Mushrooms	Mean length of the stipe (cm)	Mean diameter of pileus (cm)	Arrangement of gills	Gregarious or solitary growth on mounds
<i>T. albuminosus</i>	11.4 (10–15)	9.6 (9–10)	Adnexed	Solitary
<i>T. badius</i>	4.24 (4.0–4.5)	1.46 (0.8–2.5)	Adnexed to free	Gregarious
<i>T. clypeatus</i>	9.4 (7.0–10)	6.4 (5.5–7)	Free	Solitary
<i>T. indicus</i>	3.26 (3.0–3.5)	3.30 (3.0–3.5)	Free	Gregarious
<i>T. microcarpus</i>	3.72 (3.5–4.0)	1.76 (1.5–2.0)	Adnexed	Gregarious

Values in parentheses are ranges.

nitrogen, C:N ratio, ash and moisture content of fungal comb before and after the growth of *T. microcarpus* (table 3).

There was a significant decrease in cellulose (5.9%) and lignin (3.5%) content of the comb, indicating their direct impact on the growth and development of *T. microcarpus*. Similarly, Batra and Batra (1979) observed decrease in cellulose content of fungal comb in which a species of *Termitomyces* was grown. Likewise, PrabhuDessai (1982) observed a decrease in cellulose and lignin content in paddy straws and maize cob substrate on which *Pleurotus sajor-cuju* (Fr), the edible mushroom was grown.

Significant decrease in carbon (11.2%) and nitrogen (2.34%) contents was observed due to degradation of substrates by mushrooms and nitrogen utilized in the form of fruiting bodies. During decomposition of substrate, the carbon that is lost reflects upon the C:N ratio. Thus, a gradual reduction in C:N ratio (1.37%) was observed in the fungal comb fragment after the growth of *T. microcarpus*.

Similarly, decrease in moisture content of 17.02% was observed in the substrate after the growth of *T. microcarpus*. Contrary to these results PrabhuDessai (1982) observed an increase in moisture content of paddy straw and maize cob after the growth of button mushroom *P. sajor-cuju*. The decrease was attributed to the effect of atmospheric temperature (28.5°C) on the substrate which was exposed to sunlight. Increase in ash content (20.15%) and decrease in fat content (0.48%) of substrate was due to the utilisation of carbon and fat during growth and development of *T. microcarpus*.

3.3 Nutritional value of *T. microcarpus*

These results have indicated that the mushroom (*T. microcarpus*) has been found to be a best source of protein and mineral content. The protein content ranged from 39.16–43.37%. In addition to high nitrogen content (9.68%), it was also rich in crude fat (4.98%), mineral matter (15.14%) and micronutrients such as calcium (155 meq.), magnesium (55 meq.), copper (101.92 ppm), iron (1254.20 ppm), manganese

Table 3. Chemical composition of fungal comb before and after growth of *T. microcarpus*.

Chemical composition	Growth (%)		Decrease/ increase (%)	Student 't' value
	Before growth	After growth		
Moisture	45.00	27.98	17.02	11.57**
Cellulose	26.60	20.70	5.90	6.936**
Lignin	17.20	13.70	3.50	6.932**
Nitrogen	2.88	2.34	0.54	5.280**
Carbon	42.62	31.42	11.20	15.100**
C:N ratio	14.84	13.47	1.37	2.297 ^{NS}
Crude fat	5.42	4.94	0.48	4.800**
Ash	23.28	43.43	20.15	15.104**

't' at 0.05 = 2.300; 0.01 = 3.355.

Significant at **1%.

^{NS}Not significant.

(54.60 ppm) and zinc (137 ppm). Similarly, Mukiibi (1973) observed high protein (27.4%), fat (4.3%) and ash (14.1%) content in the same species. Adriano and Cruz (1933) observed a high calcium and iron content, with 217 and 52 mg per 100 g respectively, in *T. albuminosus*.

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Age-correlated tissue preferences of *Heliothis armigera* (Hubner) and *Spodoptera litura* (F) with special reference to phenolic substances

R S ANNADURAI, S MURUGESAN and R SENRAYAN

Entomology Research Institute, Loyola College, Madras 600 034, India

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Abstract. Age correlated tissue preferences of *Heliothis armigera* (Hubner) and *Spodoptera litura* (F) were investigated on *Gossypium hirsutum*, *Abelmoschus esculentus*, *Ricinus communis* and *Cajanus cajan*. The food utilization and reproductive potential of these two noctuids are correlated with the host plant nutritional and non-nutritional phenolic substances, separated and characterised through thin-layer chromatography and UV spectra. The non-nutritional phenolic substances such as phloroglucinol, resorcinol, protocatechuic acid, gallic acid, pyrogallol and vanillic acid tend to vary within the host plant parts as well as in different host plant species.

Keywords. *Spodoptera litura*; *Heliothis armigera*; age-correlated tissue preferences; non-nutritional phenolic substances.

1. Introduction

Plants vary greatly in their food value for different insects and the host plant specificity is based on the insect's nutritional requirements. The nutritional quality of plant tissues does not differ considerably qualitatively, but varies quantitatively. Fraenkel (1959) suggested that all primary nutrients for insects are found in all higher plants, but the attractancy and repellency are governed by the elaborated secondary plant products. The distribution of the secondary plant products particularly phenolic substances tend to vary in different host plant tissues. Reese and Beck (1976), Carter and Lyman (1969) and Van Sumere (1975) have indicated the allelochemical effect of various phenolic substances. The influence of nutritional factors coupled with the non-nutritional phenolic substances on food preference, food utilization, growth and reproduction of two economically important noctuids viz. *Spodoptera litura* (F) and *Heliothis armigera* (Hubner) are presented in this paper.

2. Materials and methods

Larvae of *S. litura* and *H. armigera* were reared from eggs collected from the field. In all experiments newly ecdysed third instar larvae were chosen and subjected to feeding experiments. Freshly excised tissues of various host plants viz. *Gossypium hirsutum* L., *Ricinus communis* L., *Cajanus cajan* (L.) Millspaugh and *Abelmoschus esculentus* (L.) Moench. were utilised for estimation of food consumption as well as biochemical components. Semi-synthetic diet was also tested for nutritional indices and reproductive potential for both the species as indicated by Ananthakrishnan *et al* (1990).

2.1 Quantitative food utilization

The gravimetric method was adopted for assessing the quantity of food utilised. Experimental larvae were separated from the stock culture and the initial weights recorded; then introduced to individual feeding chambers where they were allowed to feed on weighed quantity of the chosen plant tissues for 24 h. At the end of the experiment, the left over food, excreta and the instars were weighed. The difference in the weight of the instar gave the weight gained during the period of study. Comparison of food utilisation was carried out by studying the growth parameters of Waldbauer (1968).

2.2 Biochemical estimations

The various host tissues were subjected to biochemical estimation for their total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956), nitrogen (Vogel 1963) and phenol (Hori 1974).

2.3 Extraction and separation of phenolic substances

About 15 g of each selected plant tissue was subjected to acid hydrolysis with 2 M HCl for 30 min. The solution was later cooled and filtered. The phenols were subsequently taken into ether and the ether extract was dried. The residue was then dissolved in ether and chromatographed on silica gel in acetic acid chloroform; ethyl acetate-benzene; and benzene-methanol acetic acid. The R_f values and spectral ranges of standard phenols and phenolic acids were compared with the eluted fractions (Harborne and Williams 1969).

2.4 Fecundity studies

In order to assess the impact of leaf quality on the fecundity of adult *S. litura* and *H. armigera*, larvae reared from the different feeding regimes were allowed to pupate separately till eclosion. Males and females emerging from similar feeding regimes were allowed to mate and the number of eggs laid till death was taken as fecundity with 10 replications.

3. Results

3.1 Age-correlated tissue preference of *H. armigera*

The differential quantitative food preferences of *H. armigera* are depicted in figure 1. Of all the host plant tissues, the mature bolls of *G. hirsutum* were consumed more (2.092 g) by the 10-day old larva of *H. armigera*. The 10-day old larva when reared on the pods of *C. cajan* consumed 1.057 g but it egested only 0.018 g. The growth rate was very high (1.35 g/day) in the case of 7-day old larva when fed with pods of *C. cajan*. *G. hirsutum* squares supported growth faster than the bolls. A comparison of the total amount of food consumed during the larval stages among the different

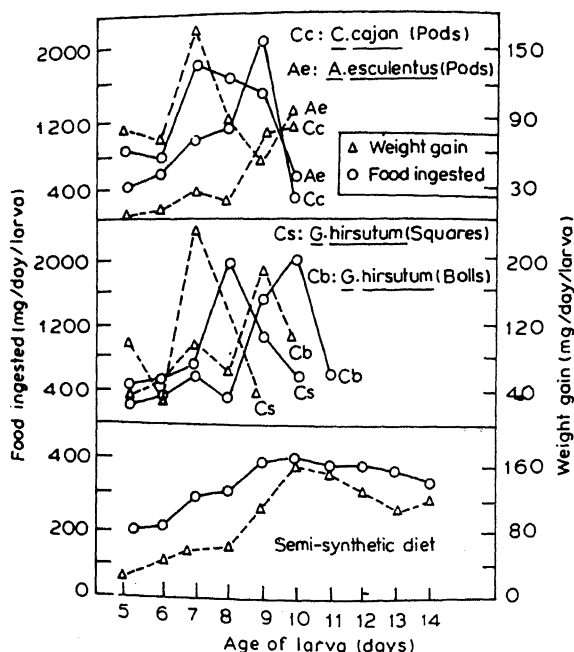


Figure 1. Host plant tissue preference of *H. armigera*.

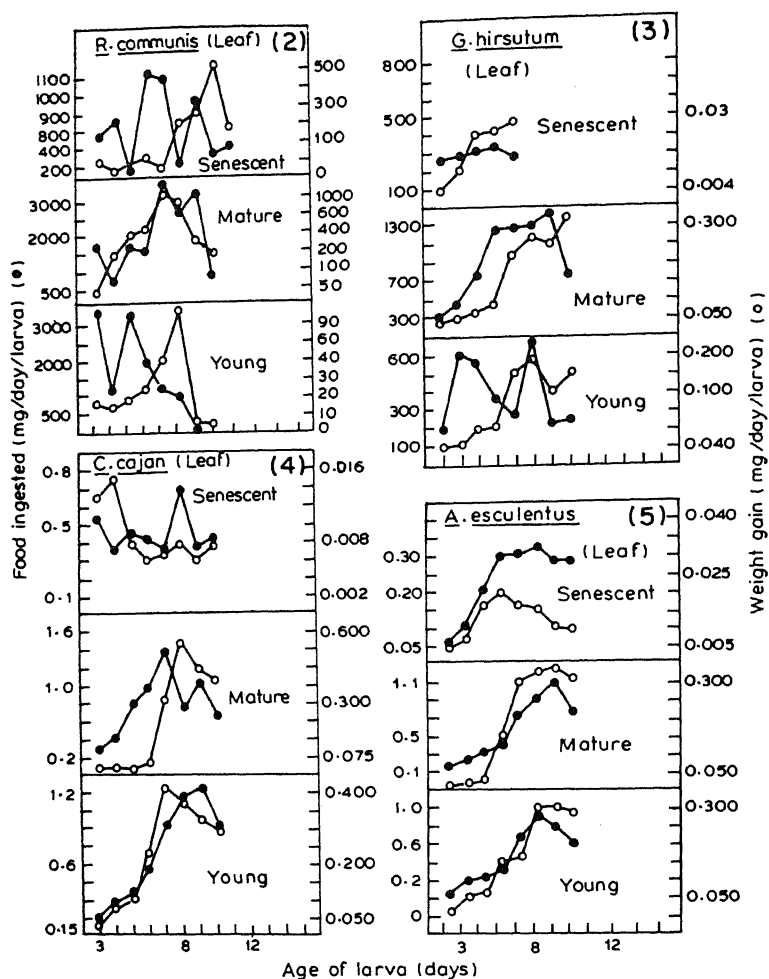
host tissues indicate some interesting results. Maximum amount of *A. esculentus* pods were utilised (7.505 g) followed by cotton bolls (5.779 g), squares (5.270 g) and *C. cajan* pods (4.345 g). However, the body weight gained was maximum only in the case of cotton bolls (0.552 g) followed by squares (0.524 g), *C. cajan* pods (0.388 g) and *A. esculentus* pods (0.365 g).

3.2 Host tissue preference of *S. litura*

Of the 4 host plants tested for *S. litura*, mature leaves of *R. communis* were preferred as compared to young and senescent leaves, the order of preference for other hosts being *G. hirsutum*, *C. cajan* and *A. esculentus*. The consumption efficiency in terms of body weight gain on all host tissues is illustrated in figures 2–5. Maximum food was consumed by 7-day old larva (3.957 g) on mature larvae of *R. communis* and the body weight gain was 1.220 g/day. By the 10th day the food consumption declined drastically (0.341 mg). In the case of mature leaves of *G. hirsutum*, the maximum quantity was utilised on the 10th day (1.146 g). Six-day old larvae consumed 1.4 g of mature leaves when compared to young and senescent leaves.

3.3 Host tissue influenced fecundity

The food plant tissue quality was clearly reflected in the reproductive capacity of the moths. Individuals maintained on *G. hirsutum* bolls laid a maximum of 1865 ± 155 (mean \pm SD) eggs with an oviposition period of 7 days. Individuals maintained on *C. cajan* pods laid a maximum of 1335 ± 128 eggs within 5 days



Figures 2-5. Host plant tissue preference of *S. litura* on (2) *R. communis*, (3) *G. hirsutum*, (4) *C. cajan* and (5) *A. esculentus*.

while 1255 ± 125 eggs were laid by individuals maintained on *G. hirsutum* squares. *A. esculentus* pods seem to be a poor quality diet since only 1210 ± 108 eggs were laid.

Figure 6 indicates the total egg output of *S. litura* when reared on the leaves of different ages of *R. communis*, *A. esculentus*, *G. hirsutum* and *C. cajan* as well as on the semi-synthetic diet. A maximum of 1966 ± 103 eggs were laid when reared on mature leaves of *R. communis*. When reared on young leaves they laid only 1744 ± 177 eggs and individuals reared on senescent leaves laid only 833 ± 112 eggs. Individuals reared on semisynthetic diet were able to lay as much as 1605 ± 127 eggs. Mature leaves of cotton, seemed to support maximum fecundity (1802 ± 130 eggs) next to *R. communis* and in this case also the young leaves were next to mature leaves (1616 ± 115 eggs). Senescent leaves yielded only 773 ± 87 eggs and the reduction is less than 50%. *C. cajan* leaves appeared almost similar to *R. communis*

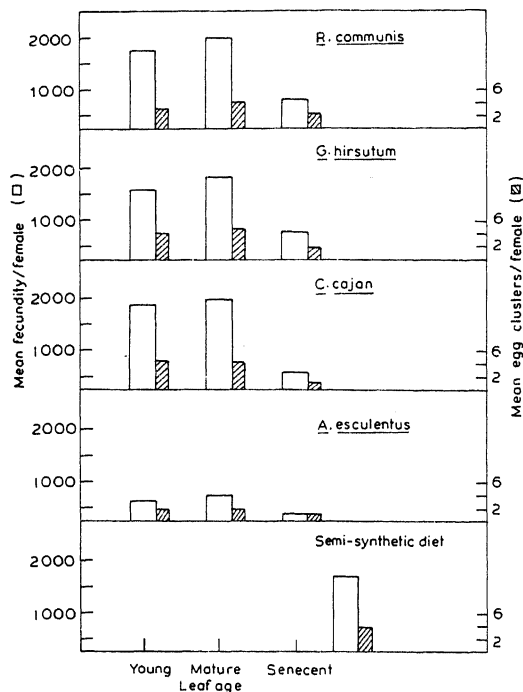


Figure 6. Reproductive potential of *S. litura* on the various host plant tissues.

deciding the egg output. However, in the case of *A. esculentus*, the egg output was reduced considerably and the maximum was attained on mature leaves (740 ± 83).

3.4 Age-correlated host tissue chemical profile

The various biochemical nutritional components such as total proteins, total carbohydrates, total nitrogen and water were analysed for the host tissues of different ages in addition to the non-nutritional plant substances such as phenolics. Table 1 provides the data relating to the concentration of various biochemical components of the host plant tissues tested. Mature leaves of *R. communis* had the highest concentration of protein (73 mg/g) and amino acids 0.46 mg/g when compared to all other host plants. The carbohydrate concentration was very high in the case of mature leaves 275 mg/g of *G. hirsutum*. Mature leaves of *A. esculentus* had a higher concentration of phenols (53 mg/g). The water/nitrogen (W/N) ratio was also assessed for determining the food plant preferences. Among the host leaves, W/N ratio was less in *R. communis*; while the ratio was the lowest in senescent leaves where the preference was also the least. Of all the hosts, *G. hirsutum* leaf tissues showed the highest W/N ratio. In order to identify the role of non-nutritional phenolic substances, extraction, separation and characterization were made of the different host plant tissues. Young *G. hirsutum* squares showed the presence of vanillic and salicylic acid while the mature bolls showed the presence of vanillic acid, phloroglucinol and protocatechuic acid. Protocatechuic acid was also identified in the young leaves of *G. hirsutum* in addition to pyrogallol. Mature

Table 1. Biochemical composition of host leaves/pods.

Host leaves/ pods	Protein (mg/g)	Carbohydrates (mg/g)	Phenols (mg/g)	Amino acids (mg/g)	N2 (%)	Water (%)	W/N (%)
<i>Ricinus communis</i>							
Young	61	241	31.6	0.238	3.70	63.0	17.10
Mature	73	265	35.3	0.464	4.25	78.0	18.40
Senescent	27	170	28.8	0.132	2.63	35.0	13.30
<i>Gossypium hirsutum</i>							
Young	36	228	23.8	0.121	2.92	70.1	24.00
Mature	58	275	41.0	0.163	3.38	88.3	26.12
Senescent	19	153	28.6	0.081	2.23	45.2	20.27
Square	43	321	18.5	0.216	3.11	75.2	24.16
Bolls	63	435	19.8	0.259	3.52	91.5	25.99
<i>Cajanus cajan</i>							
Young	32	167	43.0	0.185	2.75	60.0	21.8
Mature	40	173	48.0	0.232	3.68	88.0	23.7
Senescent	18	081	31.3	0.106	2.25	41.0	18.2
Pods	51	285	21.6	0.213	3.81	93.0	24.27
<i>Abelmoschus esculentus</i>							
Young	29	152	46.2	0.286	3.02	65.0	21.52
Mature	35	168	53.0	0.358	3.63	85.0	23.41
Senescent	14	109	37.3	0.220	2.51	48.0	19.12
Pods	43	187	28.6	0.223	3.76	89.0	23.75

leaves contained gallic acid, hydroquinone and protocatechuic acid. Senescent leaves showed the presence of 2-methyl resorcinol, hydroquinone, phloroglucinol and resorcinol (table 2). The R_f values and spectral maxima of the substances identified are presented in table 3.

In the mature pods of *A. esculentus* the presence of vanillic and protocatechuic acids were noticed. The 3-leaf stages showed the presence of protocatechuic acid, while young and mature leaves possessed gentisic acid in addition to resorcinol. The mature pods, young leaves, mature leaves and senescent leaves of *C. cajan* had phloroglucinol, protocatechuic acid, 2-methyl resorcinol and resorcinol respectively (table 2).

4. Discussion

S. litura and *H. armigera* when tested for their age correlated tissue preference, fecundity and biochemical interaction indicated that the larvae of *S. litura* showed a higher food intake and better growth rate when fed on the mature leaves of *R. communis*. However larvae of *H. armigera* showed a higher food intake and growth when fed on the cotton bolls when compared with the different leaf stages. Pretorius (1976), based on the net reproductive rates of *H. armigera*, reported that the best food was cotton buds and leaves, followed by groundnut leaves, sunflower heads, green bean, rose petals, potato leaves, lucerne leaves, grains, sorghum

Table 2. Phenolic substances identified in the host tissues of *H. armigera*.

Host plants	Tissue	Phenolic substance
<i>Gossypium hirsutum</i>	Young squares	Vanillic acid, salicylic acid
	Mature bolls	Vanillic acid, phloroglucinol, protocatechuic acid
	Young leaves	Pyrogallol, protocatechuic acid
	Mature leaves	Gallic acid, hydroquinone, protocatechuic acid
	Senescent leaves	2-Methyl resorcinol, hydroquinone, phloroglucinol, resorcinol
<i>Abelmoschus esculentus</i>	Mature pods	Vanillic acid, protocatechuic acid
	Young leaves	Protocatechuic acid, gentisic acid
	Mature leaves	Protocatechuic acid, resorcinol, gentisic acid
	Senescent leaves	Protocatechuic acid
<i>Cajanus cajan</i>	Mature pods	Phloroglucinol
	Young leaves	Protocatechuic acid
	Mature leaves	2-Methyl resorcinol
	Senescent leaves	Resorcinol
Benzene derivatives		Benzoic acid derivatives
Resorcinol—1,3-dihydroxy benzene		Salicylic acid—2-hydroxy benzoic acid
Hydroquinone—1,4-dihydroxy benzene		Gentisic acid—2,6-dihydroxy benzoic acid
Phloroglucinol—1,3,5-trihydroxy benzene		Gallic acid—3,4,5-trihydroxy benzoic acid
Pyrogallol—1,2,3-trihydroxy benzene		Vanillic acid—4 hydroxy-3-methoxy benzoic acid
		Protocatechuic acid—3,4-dihydroxy benzoic acid

Table 3. Characterization of phenolic substances.

Phenol	Solvent system (mobile phase)	R_f value ($\times 100$)	Spectral maxima (nm)
Benzene derivatives			
Resorcinol—1,3-dihydroxy benzene*	HOAc-CHCl ₃	19	291.2
Hydroquinone—1,4-dihydroxy benzene	Benzene-MeOH-HOAc	32	297.6
Phloroglucinol—1,3,5-trihydroxy benzene*	EtOAc-CHCl ₃	47	247.0
Pyrogallol—1,2,3-trihydroxy benzene	Benzene-MeOH-HOAc	19	260.8
Benzoic acid derivatives			
Salicylic acid—2-hydroxy benzoic acid*	EtOAc-benzene	80	235.0
Gentisic acid—2,6-hydroxy benzoic acid	Benzene-MeOH-HOAc	42	332.0
Gallic acid—3,4,5-trihydroxy benzoic acid	EtOAc-benzene	42	272.0
Vanillic acid—4-hydroxy-3-methoxy benzoic acid	Benzene-MeOH-HOAc	71	256.0
Protocatechuic acid—3,4-dihydroxy benzoic acid	HOAc-CHCl ₃	19	248.4 254.5

*Compounds characterized based on the best separation achieved among 3 different solvent systems.

panicles, young maize cobs and tomato leaves. Adult production on *G. hirsutum* was about 4 times more compared to maize. However Sparks *et al* (1971) reported that corn particularly a multieared sweet corn, produced 4 times as many corn earworm on *G. hirsutum*. Scriber and Feeny (1979) and Scriber (1978) have concluded that chemical factors correlated with plant growth account for the major portion of the variation in larval efficiency. Scriber (1978) reported no difference in consumption rate, assimilation, efficiency of utilization of plant biomass of *S. eridania* when fed with acynogenic and cynogenic leaves of *Lotus corniculatus*. Larvae of *S. litura* and *H. armigera* when fed with the different host plant tissues of

different ages show clear variation with respect to growth, food consumption and fecundity. These differences may be attributed to the differential biochemical composition of the host tissues particularly the phenolic substances. In the present study, it was possible to characterise the various phenolic substances in the different host tissues. Reese and Beck (1976) have indicated that resorcinol inhibited ingestion and growth but did not inhibit pupation or pupal weight of the black cutworm, *Agrotis ipsilon*. Phloroglucinol inhibited survival, growth and pupation. It inhibited growth by reducing the efficiency of conversion of assimilated and ingested food. The presence of phloroglucinol in the mature bolls of *G. hirsutum* and mature pods of *C. cajan* may account for the higher preference of *H. armigera* to these tissues since phloroglucinol stimulates growth, survival and pupation at low concentrations even though it is a harmful stress agent apparently due to hormoligosis (the phenomenon in which harmful quantities of many stress agents may be stimulating) (Luckey 1968). The presence of resorcinol in the leaves of *C. cajan* and *G. hirsutum* may be the reason for the reduced rate of ingestion of the senescent leaves in both the species. It is possible that the presence of any one of these substances singly or in combination with other substances may play an attractant or repellent role and it is crucial to indicate their role before ascertaining their absolute quantities in plant tissues.

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Hatching patterns in the silkworm *Bombyx mori* L. (PM × NB4D2) under different photoperiodic combinations

N SIVARAMI REDDY*† and K SASIRA BABU

Department of Zoology, SV University, Tirupati 517 502, India

*Present address: Central Sericultural Research and Training Institute, Srirampuram, Manandavadi Road, Mysore 570 008, India

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Abstract. Hatching pattern in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions was studied. Hatching was predominantly diurnal under natural solar day conditions (LD 12:12). The observed LD pattern prevailed under all the photoperiodic conditions. Hatching confined to a single day under all the short-day conditions as also LD 12:12 and DD conditions. All the long-day photoperiodic conditions, as also continuous light (LL), resulted in broadening of the hatching peaks with reduced amplitudes, with extended hatching for two days apart from long hatching durations with more hatching on the second day. The hatching rhythmicity seems to be under circadian control. Maximum hatching was observed under LD 11:13 photoperiodic combination. Shortest hatching duration was observed under LD 11:13 condition.

Keywords. Photoperiod; hatching; circadian control; *Bombyx mori*.

1. Introduction

Under light-dark schedules of natural day (\leq or \geq 24 h; Saunders 1978, 1982) animals exhibit rhythmic fluctuations (Solberger 1965). Embryonic diapause in response to photoperiods characterizing short-day nature (Kogure 1933), growth rates (Hirasaka and Koyama 1970, 1972; Sivarami Reddy *et al* 1984) and various other phenomena showing cyclical fluctuations in *Bombyx mori* L. were reported. *B. mori* was reported as a short day insect (Kogure 1933; De Wilde 1962; Danilevskii 1965; Lees 1968; Beck 1980; Saunders 1982), while Shimizu (1982) showed that an artificial diet produced long day characteristics for individuals that passed through embryonic diapause in the next generation. The present investigation describes rhythmic pattern in hatching under different photoperiodic combinations in *B. mori* (PM × NB4D2 a hybrid of pure Mysae, a multivoltine and NB4D₂, a bivoltine).

2. Materials and methods

Disease free layings (DFLs, each DFL consists of 350–450 eggs laid by a single silkmoth on a single day) of the silkworm, *B. mori* (PM × NB4D2) were procured, third day after oviposition, for the study from the Government Silk Farm, Palamaner, Andhra Pradesh. The DFLs were transported to the laboratory during evening hours and immediately spread in the rearing trays. The same day, DFLs were introduced till hatching, to normal day (LD 12:12), DD (continuous dark

†To whom all correspondence should be addressed.

condition), LL (continuous light) and other different photoperiodic regimens, starting from LD 4:20 (short-day) to LD 20:4 (long-day). For all the photoperiodic combinations the light phase (photophase) (around 50 Lux) of the LD cycle commenced from 0600 h (for example for LD 12:12 condition the photophase was from 0600–1800 h and the scutophase spanned from 1800–0600 h). Optimum temperature ($25 \pm 1^\circ\text{C}$) and Rh ($80 \pm 5\%$) (Krishnaswami 1986) was maintained in the laboratory all through the experimentation. Five DFLs were kept under each photoperiodic conditions. Rhythmic pattern in the hatching was studied under these photoperiodic schedules. Precise timings, in hours, for hatching was determined and recorded.

3. Results

Hatching was predominantly diurnal with about 95% of hatching observed at early hours of the light part of the natural LD cycle (LD 12:12) (figure 1J). The observed LD 12:12 pattern prevailed for hatching under all the other photoperiodic conditions also. For all the short-day photoperiodic combinations, the hatching was confined to a single day. However, under all the long-day photoperiodic combinations the hatching was observed on the next (second) day also. The occurrence of peaks, in hatching was observed moving towards the early hours of the photophase under short-day conditions and the same under long-day conditions towards late hours (figure 1) with the hatching spreading over long duration of time. Further, the hatching peaks under photoperiodic combinations longer than 12 h of light has been observed broadening with less amplitudes. Except under LD 13:11 and 18:6, where hatching is more on the first day itself, hatching was more on the second day under all other long-day combinations (figure 3). Under continuous conditions (LL and DD) negligible and absolutely no hatching respectively was observed on the first day (figures 1A, S and 3). Under all the photoperiodic combinations, the hatching was predominantly diurnal. Interestingly, the magnitude of first day hatching was more at the peak hours under short-day conditions as also DD when compared to that under long-day and continuous light conditions (figures 1, 2) indicating broadening of peaks.

Total hatching was highest under LD 11:13 condition (99%) (figure 4). Hatching under LD 10:14 (98%) was second to LD 11:13 condition. Total hatching, under all the conditions was equal to or more than 90%. However, it was less than 95%, observed for LD 12:12 condition, under extreme long or short photoperiodic conditions (figure 4).

Hatching duration of the silkworm eggs (from initiation to the completion of hatching) (figure 5) had been very minimum, ranging from 1–3 h for the eggs under short-day conditions as also LD 12:12 condition. Lowest hatching duration (≈ 1 h) was observed under LD 11:13 photoperiodic condition. Interestingly, the hatching under all the long-day photoperiodic conditions was prolonged, the duration being more than 24 h. Under DD condition, however, eventhough the hatching was observed only on the second day the duration was about 5 h. On the other hand, the same under LL condition was around 24 h apart from occurring for two consecutive days.

4. Discussion

The rhythmic pattern in hatching of the eggs has been studied only in limited

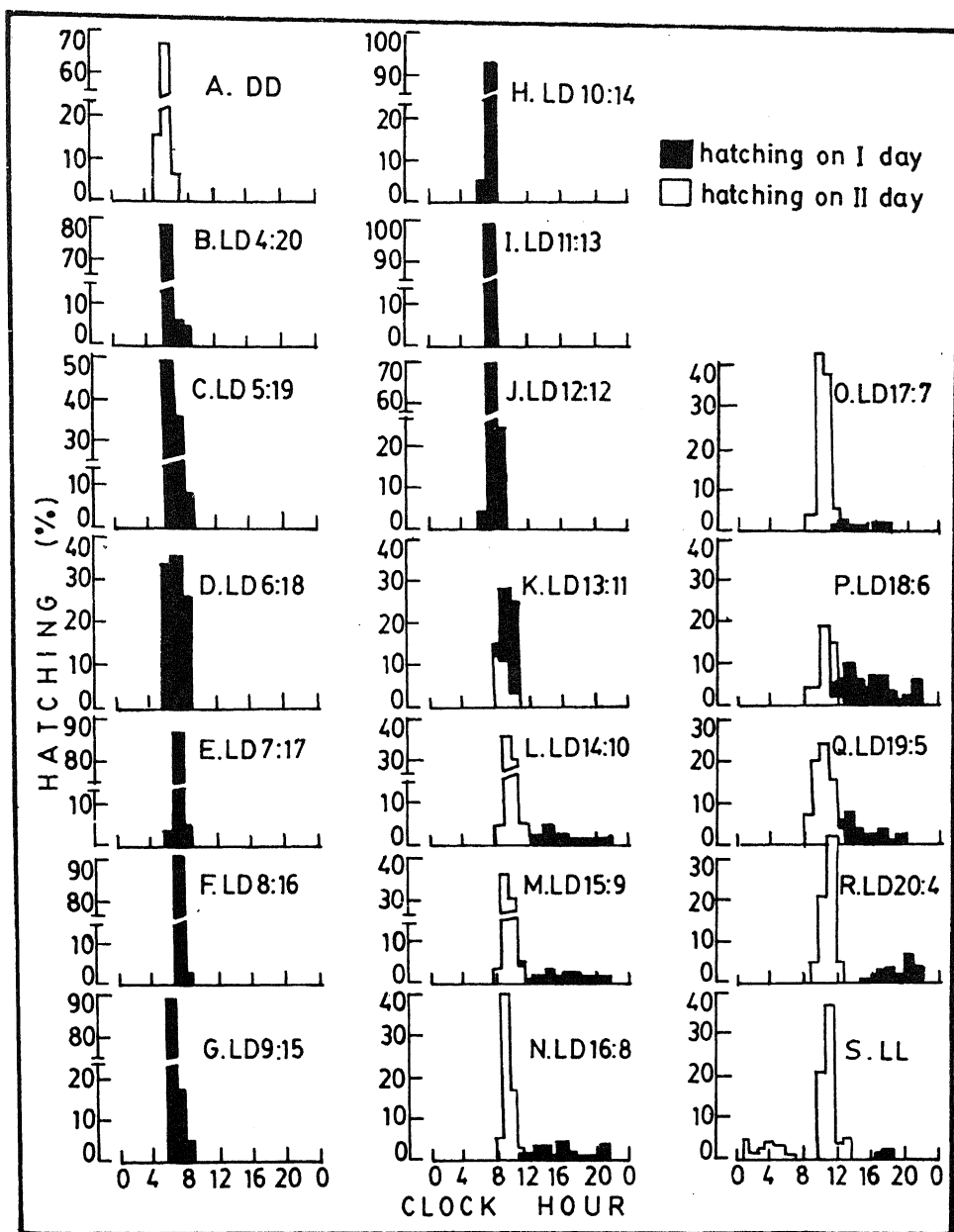


Figure 1. Distribution of hatching (%) in the silkworm, *B. mori* (PM x NB4D2) under different photoperiodic conditions; continuous dark (DD) (A), short-day (LD 4:20 to 11:13) (B-I), natural day (LD 12:12) (J), long day (LD 13:11 to 20:4) (K-R) and continuous light (LL) (S). Note hatching on single day under DD to LD 12:12 with shorter hatching duration and phase moving towards early hours of photophase (A-J) and two days under LD 13:11 to LL with broadening of hatching peaks, prolonged hatching durations and phase moving towards late hours of the photophase (K-S)

insects. In *Pectinophora gossypiella* (Minis and Pittendrigh 1968) eggs under continuous photoperiodic (DD/LL) conditions were arrhythmic in hatching but

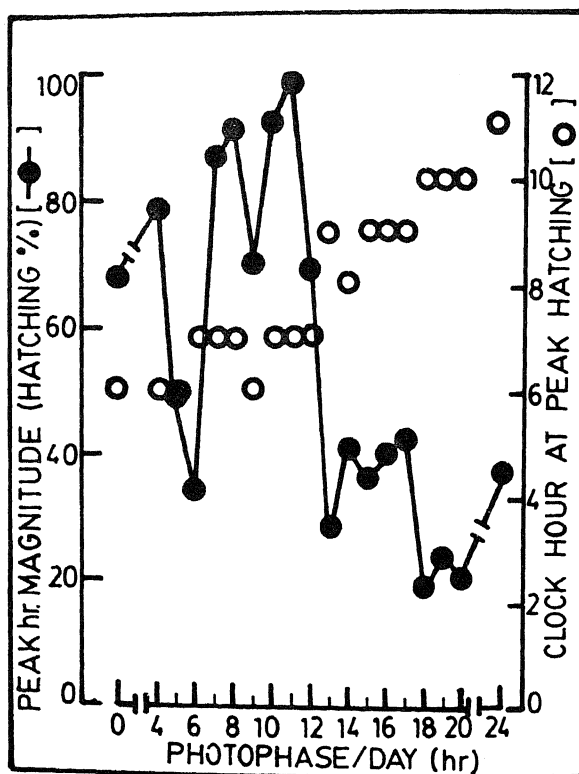


Figure 2. Hatching (%) in *B. mori* (PM \times NB4D2) at peak hour and clock hour at peak hatching under different photoperiodic conditions. Note maximum hatching for LD 11:13 and 10:14 and less hatching for extreme long/short-day photoperiodic conditions including continuous conditions (DD and LL). Also note phase delay for long-day and phase advance for short-day photoperiodic conditions.

under LD 12:12 condition there occurred a distinct rhythm, with hatching peaks just before dawn. For *Aedes* sp., hatching was observed as a direct response to environmental factor (Gillett 1955). Nayar (1967), however, reported that the preconditioned eggs of *Aedes taeniorrhynchus* hatch at any time of the day with 15 min of immersion in deoxygenated water. Rhythmic patterns in eclosion, oviposition, and hatching, as effected by photoperiods in silkworm have been reported (Yamaoka and Hirao 1975; Yamaoka *et al* 1976; Anantha Narayana 1980). Anantha Narayana *et al* (1978) explained the hatching peak in Mysore race (PM) of *B. mori* to be very close to dawn under alternating cycles of light and dark, the system links the 'light-on' as the synchronizing signal. In the present study also, hatching in *B. mori* under short-day photoperiodic regimes has been observed very close to dawn (light-on). It could be inferred that the occurrence of hatching during early dawn hours might be due to the relatively high humidity in the micro/macro environment which minimizes 'the risk' of desiccation as Pittendrigh (1966) demonstrated for eclosion in *Drosophila*.

Pupal eclosion rhythm in *D. pseudoobscura* (Pittendrigh 1966) suggests the existence of a self-sustained oscillator which partitions a mixed-age population into

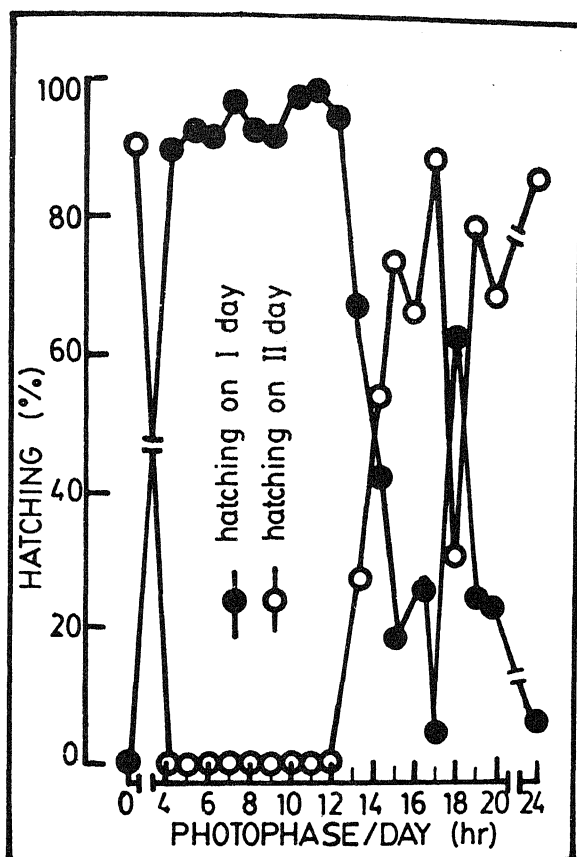


Figure 3. Hatching (%) in *B. mori* (PM×NB4D2) under different photoperiodic conditions. Note single day hatching under continuous dark (DD) and short-day combinations (LD 4:20 to 11:13) as also normal day (LD 12:12) conditions. On the other hand, hatching is less on the first day and more on the second day for all the long-day (LD 13:11 to 20:4) as also continuous light (LL) conditions, apart from prolonged hatching for two days.

daily active peaks; certain hours of the day constitute 'forbidden zones' and certain other hours of the day are 'allowed zones or gates', and are directed by the clocks. Works by Skopik and Pittendrigh (1967) and Pittendrigh and Skopik (1970), indicate that if the animals are not at the 'correct' morphogenic state to utilise one particular allowed zone or gate, they are required to remain waiting till the onset of next gate for utilisation, the intervening hours consisting a 'forbidden zone'. The gate or forbidden zones recur with circadian frequency after the LL/DD transfer. The gating of certain developmental stages by such a mechanism is probably ubiquitous in insect population rhythms (Saunders 1982). It is evident, for example, in pupal eclosion of *Antheraea pernyi* (Truman 1971) egg hatching (Minis and Pittendrigh 1968) and oviposition in *Pectinophora gossypiella* (Pittendrigh and Minis 1964) and in the oviposition rhythm of *Oncopeltus fasciatus* (Rankin *et al* 1972).

Early investigations on the effect of LL on the eclosion rhythm in *D. pseudoobscura* (Pittendrigh and Bruce 1957; Chandrashekar and Lohar 1969)

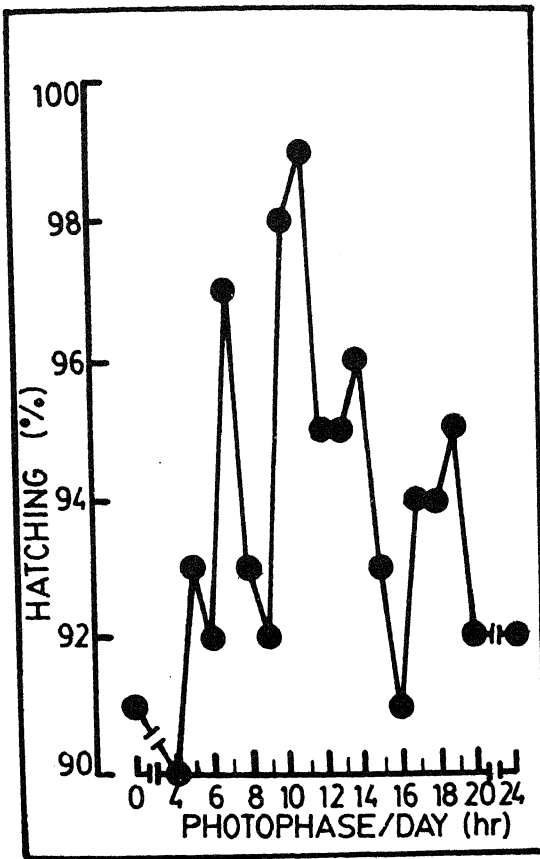


Figure 4. Total hatching (%) in *B. mori* (PM x NB4D2) under different photoperiodic combinations. Note maximum hatching under LD 11:13. Hatching under all the other photoperiodic conditions was <95% but \geq 90%.

showed fairly rapid damp-out to eventual arrhythmicity under LL condition. The results given in Pittendrigh (1960, 1966) implies that, photoperiods longer than 12 h not only damp-out the oscillation but hold the oscillations (in each individual in the population) in the same fixed stage which corresponds to that at circadian time 12, so that, following entry or re-entry into the dark, all the oscillations in the population resume their motion at the same phase. Saunders (1982) is of the view that although the overt rhythm may be damped out in LL, constant light of quite high intensity does not suppress the motion of the underlying pace maker (although it may reduce its amplitude) as he (Saunders 1979) observed broadening of eclosion peaks in *Sarcophaga argyrostoma* with higher intensities of continuous light.

Though experimentations were not carried with mixed-age populations in the present investigation, it is clear that the underlying driving oscillation in the hatching of *B. mori* is under the control of the circadian system as hatching follow the gating pattern. Further, hatching peak is broadening with photoperiods more than 12 h as also under LL conditions though not expressing the damp-out situation of the rhythmicity since, the number of photoperiodic cycles experienced

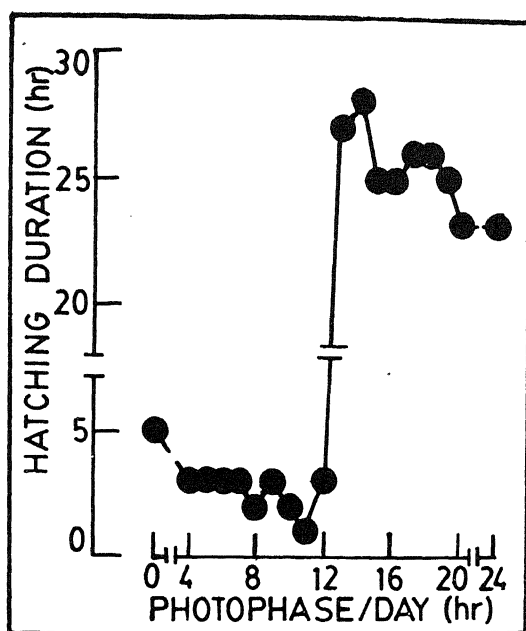


Figure 5. Hatching in *B. mori* (PM \times NB4D2) under different photoperiodic conditions. Note less hatching duration under DD and short-day photoperiodic conditions (LD 4:20 to 11:13) as also LD 12:12. On the other hand, hatching duration is more for all long-day combinations as also LL.

by the experimental population is limited (7–8 cycles). Such instances have been widely reviewed by Saunders (1982). A state of arrhythmicity in hatching patterns has been demonstrated in *B. mori* by Anantha Narayana *et al* (1978) after exposure to LL condition from its previous generation.

Eversince the report of Kogure (1933), this species of silkworm was considered as a short-day insect (De Wilde 1962; Danilevskii 1965; Lees 1968; Beck 1980; Saunders 1982). However, Shimizu (1982) demonstrated that with artificial diet, the embryonic diapause in the next generation characterized long-day photoperiodic regime. On a overview, the short-day schedule, in the present investigation, resolved maximum hatching with the shortest hatching duration in *B. mori* offering a chronobiologic approach for a greater return of commercial product (Tucker and Ringer 1982; Sivarami Reddy *et al* 1984).

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Population dynamics of *Brachionus patulus* Muller (Rotifera) in relation to food and temperature

S S S SARMA[†] and T RAMAKRISHNA RAO*

Department of Zoology, University of Delhi, Delhi 110 007, India

[†]Present address: Department of Animal Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. The combined effects of food (*Chlorella*) and temperature on the population growth of a laboratory clone of the rotifer *Brachionus patulus* were studied. The intrinsic rate of increase (r) as well as the maximum densities achieved were a function of temperature. While the r was negative at 15°C regardless of food level, its magnitude was dependent on food level at medium (25°C) and high (35°C) temperatures, indicating a significant temperature \times food interaction. The seasonal variations in the abundance of *Brachionus patulus* in the plankton of a regularly sampled pond are explained in terms of the observed food-temperature effects on the survival and reproduction of the laboratory population.

Keywords. Rotifera; *Brachionus patulus*; population dynamics; food; temperature.

1. Introduction

A thorough knowledge of factors influencing the dynamics of rotifer populations is essential not only for understanding zooplankton community organization in natural freshwater bodies (Dussart *et al* 1984; Herzig 1987), but also for maximizing production of rotifers in aquaculture where they are offered as the first live food for cultured fish and crustaceans (Gatesoupe and Luquet 1981; Lubzens 1987; James and Abu-Rezeq 1989). Another area for application of this knowledge is aquatic ecotoxicology where certain parameters of rotifer population dynamics have been considered ideal for the bioassay of sublethal levels of pollutants (Halbach *et al* 1981; Halbach 1984). Although there have been field and laboratory studies on the effects of temperature and food levels on rotifers (Edmondson 1965; Halbach 1970; Pourriot and Rougier 1975; Hofmann 1977; Rothhaupt 1985; Galkovskaja 1987), very few considered the combined effects of these two factors, particularly for species inhabiting tropical freshwaters (Duncan 1984). Population dynamics, as Halbach *et al* (1981) put it, acts as 'magnifying glass' for small but significant changes in the life history traits of individual organisms, changes which are summed over thousands of individuals and find detectable expression at the population level. Recently we examined the interaction of food and temperature in influencing certain life history traits of *Brachionus patulus*, a ptychoplanktic rotifer commonly found in ponds (Sarma and Rao 1990). This communication reports the combined effects of food and temperature on the population growth of *B. patulus* and attempt to relate the laboratory findings to certain seasonal variations in its abundance in nature.

*To whom all correspondence should be addressed.

2. Materials and methods

2.1 Laboratory studies

The experimental animal *B. patulus* was cultured in the laboratory using a starter culture of amictic females cloned from a single individual collected from local plankton. The rotifers were fed with single-celled alga *Chlorella*, mass-cultured in the laboratory, and maintained in a BOD incubator at $27 \pm 2^\circ\text{C}$ under diffuse fluorescent illumination. Based on a series of preliminary tests, temperature levels of 15° , 25° and 35°C , and food levels of 1, 2 and 4×10^6 cells (*Chlorella*)/ml, were chosen for the final tests. Earlier dynamics experiments (Sarma 1987) indicated that very low (<10 /ml) initial densities (N_0) could lead to chance extinction of population under certain test conditions (particularly low temperatures). We therefore selected a fairly high N_0 of 50 ± 5 /ml for the tests, after experimentally confirming that the magnitude of N_0 did not significantly influence the reproductive rates and carrying capacities achieved by the rotifer under optimal conditions (Sarma and Rao 1987).

Chlorella in the log phase of their growth were harvested from 10 l culture bottles, centrifuged (5 min at 800 g), rinsed and resuspended in distilled water. Desired food levels were obtained by serial dilution of this stock solution, the *Chlorella* density of which was measured using a hemocytometer. Glass beakers (25 ml) with 20 ml medium with *Chlorella* at one of the test densities (1 , 2 or 4×10^6 cells/ml) were maintained at each of the experimental temperatures (15° , 25° and $35^\circ \pm 1^\circ\text{C}$) in thermostatically controlled water baths under continuous illumination. Three replicates were set up for each treatment (food-temperature combination). Rotifers at a density of 50 ± 5 /ml were introduced into each of the 27 beakers (3 food levels \times 3 temperatures \times 3 replicates). The initial population in each case included neonates, non-egg bearing adults and ovigerous adults in approximately equal proportions, with an age composition typical of a growing population in stock cultures. Although *B. patulus* in the stock cultures bred mostly parthenogenetically, occasionally males and mictic females with eggs were observed during the tests but were excluded from population counts in view of their very low frequency (0.2–0.4%).

Rotifer densities in each beaker were measured at 24 ± 2 h intervals by taking 2–3 aliquot samples of 1 ml each and counting rotifers under a stereozoom binocular microscope at 10 – $20\times$ magnification. In addition, the numbers of ovigerous adults, neonates and eggs (including those detached from parent body) were recorded. After a 3-week period, all beakers maintained at 15° and 35°C were shifted to the intermediate temperature (25°C) and observations continued for another week.

The intrinsic rate of natural increase (r) in *B. patulus* was calculated for the exponential phase of population growth by using the formula $r = \ln N_t - \ln N_0 / t$ (Poole 1974). Where N_0 = initial density and N_t = density after time t (in days).

2.2 Field observations

Field sampling of plankton was undertaken over one year period to examine the relation between water temperature and population densities of *B. patulus* in nature.

For this study, a small (0.1 ha), perennial, oligotrophic pond adjacent to the University campus was chosen. Plankton samples were collected from the pond at 15 day intervals by filtering 50 l of water from the littoral-benthic regions through a nytex sieve (pore size: 53 μ m). The collected plankton was concentrated and preserved in 5% formalin for later analysis. Water temperature was recorded at the sampling time. In the laboratory, *B. patulus* densities were estimated by the method described in the preceding section. The frequency of neonates and egg-bearing adults in each sample was also recorded.

3. Results

3.1 Laboratory observations

In the laboratory study, both food and temperature had a significant effect on population growth in *B. patulus* (figure 1). At 15°C, where food effects were not significant, the densities declined sharply in the initial stages, either reaching an equilibrium or showing only a marginal increase subsequently. The average number of eggs/♀ at this temperature was however higher than at 25° or 35°C. When after 3 weeks the temperature was raised to 25°C, there was a rapid increase in the rotifer population density.

The population growth at 25° and 35°C showed clear, food-related trends. Although the growth pattern departed substantially from a sigmoid growth curve, a distinct peak indicating carrying capacity (K), followed by a crash and initiation of a new growth cycle could be noticed (figure 1). The maximum density achieved was influenced significantly not only by temperature and food levels independently but also by their interaction ($P < 0.001$, F -test, 2-way ANOVA). Thus at high food levels, the magnitude of population increase (over the initial density) was nearly 9.6 times at 25°C, but only 4.2 times at 35°C (figure 2). Low food levels (1×10^6 cells/ml) that contributed to a substantial increase in population density at 25°C, could not sustain population beyond 12 days and could not initiate new growth cycle following a crash at 35°C. Shifting of test individuals from 35–25°C resulted in a rapid increase in population size. The average number of eggs/♀ recorded each day was generally lower at 35° than at 25°C. The intrinsic rate of increase (r) was negative at 15°C regardless of food level (figure 3). At 25° and 35°C, food level had a significant effect on r ($P < 0.01$, F -test, 2-way ANOVA). Although between 25° and 35°C, the temperature effect on r was not significant ($P \sim 0.07$), the food \times temperature interaction was ($P < 0.01$).

3.2 Field observations

Summer temperatures of the pond water were 30–35°C while winter temperatures were in the range of 14–17°C. Maximal densities of *B. patulus* were recorded during Sept.–Oct. period when water temperatures were $26 \pm 1^\circ\text{C}$ (figure 4). A declining trend starting in December continued till March after which no *B. patulus* were represented in plankton collections for nearly 3 months (April–June). During a greater part of this period the pond temperatures were $> 30^\circ\text{C}$. *B. patulus* reappeared in plankton samples starting with July and continued with an increasing trend. On the average, about 30% of the individuals of *B. patulus* in the field

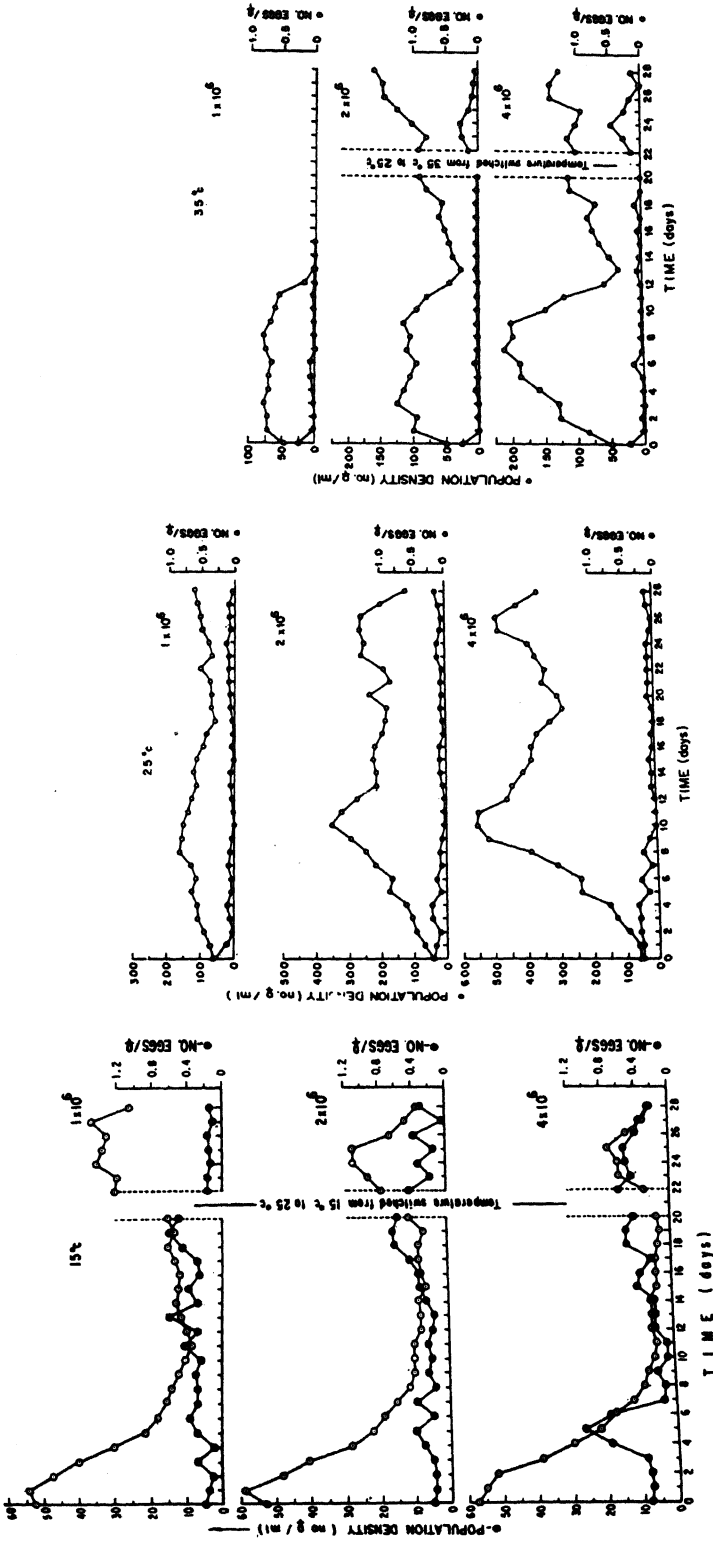


Figure 1. Population growth (○) and number of eggs/♀ (●) in *B. patulus* grown at 3 food levels at 15°, 25° and 35°C. Test populations held at 15° and 35°C were shifted to 25°C separately after 21 days. Each point represents the mean of 3 replicates.

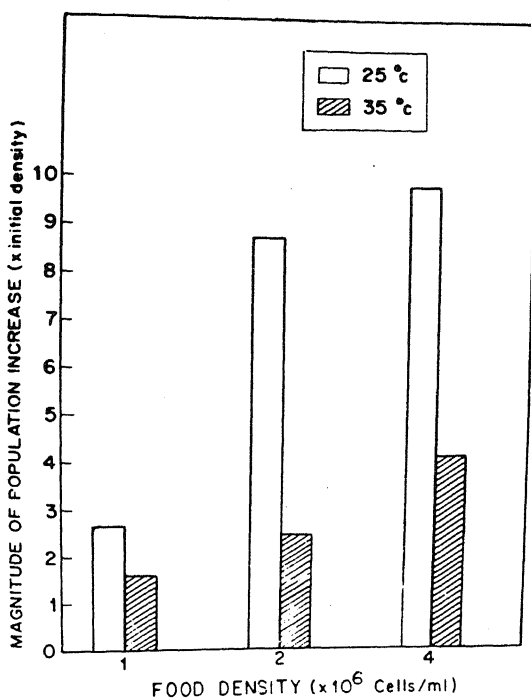


Figure 2. Magnitude of population increase (maximum density reached/initial density) as a function of food level in *B. patulus* held at 25° and 35°C. Values shown are the mean of 3 replicates.

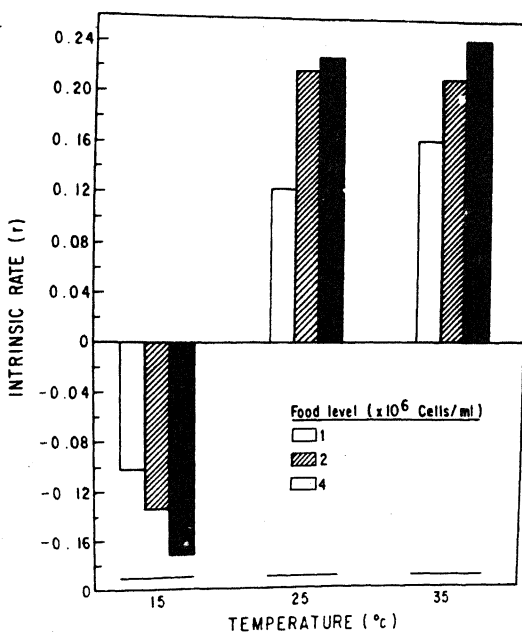


Figure 3. The intrinsic rate of increase (r) in *B. patulus* grown under different food-temperature conditions. Values shown are the mean of 3 replicates.

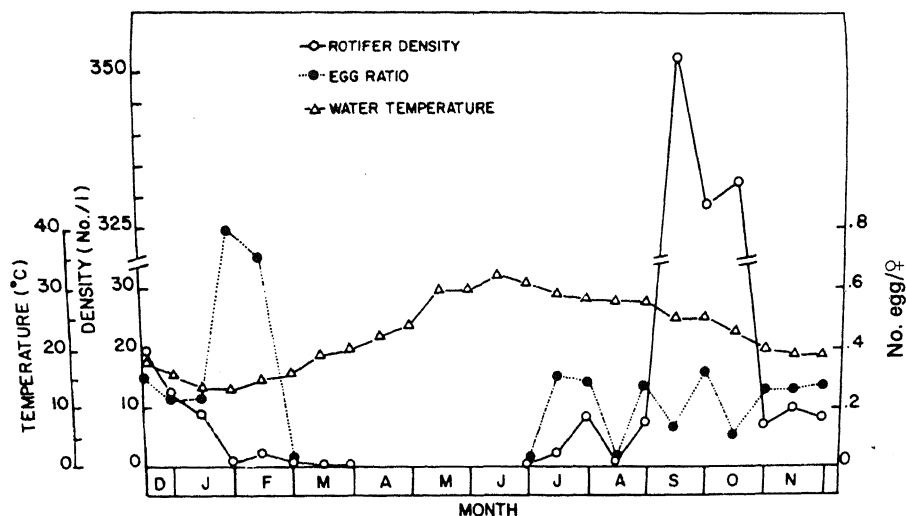


Figure 4. Seasonal variation in abundance (○) and egg ratio (●) of *B. patulus* in a pond sampled at fortnightly intervals. Also shown (△) is the surface water temperature recorded at each sampling time.

samples were ovigerous. The lowest frequency of egg-bearers was recorded in summer months and the highest in winter months (figure 4).

4. Discussion

The pattern of population growth in *B. patulus* did not follow faithfully a typical Verhulst-Pearl logistic model observed by others for different brachionid species (Halbach and Halbach-Keup 1974; Halbach 1979; Rothhaupt 1985; Walz 1987). In particular, the absence of a clearly defined lag phase in our study is probably due to the high initial densities used in the tests. Certain irregularities observed in the later growth phase could be attributed to the fact that the starter group in our study were not all of the same age class but included neonates, juveniles and ovigerous adults.

The equilibrium density (K) achieved by rotifer populations is a function of the quantity of food available (Halbach 1970) while the duration of each cycle and the amplitude of oscillations around K are known to be temperature-dependent (Halbach 1979). Temperature influences egg developmental time (Duncan 1983; Herzig 1983; Sarma and Rao 1990) and incorporation of developmental time lags into logistic growth model has indeed been shown to generate oscillations in population density around K (Krebs 1985).

The growth patterns, maximum densities achieved and intrinsic rate of increase for *B. patulus* in our study were all influenced by temperature and food levels; in many cases the temperature \times food interaction was a significant component of the overall effect. Except at 15°C, the magnitude of effect of one factor on the population parameters was dependent on the level of the other factor acting in concert; for instance, the maximum densities achieved by the rotifer at 25° and 35°C

were directly related to the food level, but the food effect was proportionately less at 25°C. In an attempt to explain similar temperature \times food interaction effects on the net reproductive rate of *B. patulus* in a life table study, we suggested that the extremely high metabolic rates at 35°C necessitate a greater portion of intake energy to be channelled into metabolism at the expense of reproductive output (Sarma and Rao 1990). The same mechanism is responsible here for the observed temperature-dependent food effects at the population level. In the present study the low food level which at 25°C did contribute to an increase in population size and to sustained population cycles, could not support the rotifer population for long at 35°C and caused its eventual extinction (figure 1). Transfer of the declining population at 35°C to the optimal 25°C resulted in a rapid increase in population density, lending further support to our metabolic explanation.

The absence of food effect on population growth at 15°C indicates that low temperature *per se* was responsible for the observed effects. At 15°C, which is probably the lower limit for *B. patulus* in nature (Jyoti and Sehgal 1979), food intake and utilization appeared to be poor. Although eggs were being produced at 15°C, the extremely long developmental time at that temperature (Sarma and Rao 1990) contributed to birth-rates far below death rates, leading to a negative r (figure 3). Prolonged developmental time must also account for the relatively high frequency of ovigerous females were observed in the population kept at 15°C as well as in natural populations in winter.

The relative abundances of many rotifer species in nature have been known to be strongly correlated with the temperature and trophic condition of the waters they inhabit (Hofmann 1977; Radwan 1980), although their absolute magnitude might be influenced by biotic factors such as invertebrate predation (Williamson 1983) and interference competition with cladocera (Gilbert 1988). In the present study, seasonal variations in the abundance of *B. patulus* in the sampled pond showed strong correlation with those in temperature (figure 4). Maximum densities of this species recorded by us and others (Vasisht and Sharma 1976; Jyoti and Sehgal 1979) were during the months when water temperatures were in the range of 22–27°C, a range that includes the temperature level we found to be optimal (25°C) in the present study. Our observations suggest that the extremely low densities of *B. patulus* observed in peak winter are probably due to low temperatures *per se*, while the low densities observed during summer months are due to the food \times temperature interaction discussed earlier. *B. patulus* being tolerant of a wide range of temperatures (12–40°C) (Arora 1966), winter temperatures of waters in Delhi probably do not affect adversely its survivorship as much as they do their reproductive output. The low fecundity, coupled with long developmental time, does not permit build-up of a large population during winter months even if food were abundant. At the other extreme, it was found that *B. patulus* can thrive at temperatures approaching 40°C, provided food concentrations were high (Sarma 1987). Because of high metabolic demands, high fecundity in summer months is possible only under high food conditions. Thus the observed decline in the abundance of *B. patulus* during periods of water temperatures above 32°C is probably due to limitingly low food levels. Compared to temperature, food factor in the life of *B. patulus* is more difficult to assess quantitatively because of wide variations in tropical waters in not only the concentration but also in the diversity of phytoplanktonic algae that are potential food to this species. Information on rotifer food concentrations in tropical

waters is rather scanty, but considering as typical of tropical freshwater bodies, Duncan's (1984) data on rotifer food levels in Parakrama Samudra, Sri Lanka, it may be suggested that during certain periods of the year, natural food levels could be low enough to be limiting for the growth of *B. patulus* populations.

Although the maximum population densities of *B. patulus* recorded in nature, except during occasional blooms, are generally low ($< 100/l$) (Jyoti and Sehgal 1979; Nogrady 1982), the densities ($\sim 550/ml$) we obtained under laboratory conditions are typical of intensive mono-cultures of brachionids (Hirata 1979; James *et al* 1983; Lubzens 1987) used as live food for fish and crustacean larvae, and attest to the paramount role of temperature and food in maximizing rotifer production in aquaculture.

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Accumulation, distribution and depuration of mercury in the green mussel *Perna viridis* (Linnaeus)

P K KRISHNAKUMAR*, R DAMODARAN and P N K NAMBISAN

School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin 682 016, India

*Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar 581 301, India

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Abstract. Accumulation, distribution and depuration of inorganic mercury in the gills, viscera, mantle and adductor muscle+foot of green mussel *Perna viridis* were investigated. Mussels were exposed to 37.5 and 75 $\mu\text{g l}^{-1}$ mercury as HgCl_2 in a static system for 4 days. The rate of accumulation was found to be the maximum in gills followed by viscera, mantle and muscle+foot. Exposed mussels were then transferred to clean seawater in a flow-through system and maintained for 25 days to study the depuration and biological halflives (TB/2) of mercury in the different body parts. After 25 days, mercury loss from the external tissues like gills and mantle was faster than from the internal tissues like viscera and muscle. Gills and mantle showed comparatively shorter TB/2 than the viscera and muscle+foot.

Keywords. *Perna viridis*; mercury; accumulation; biological halflife.

1. Introduction

Accumulation of highly toxic metals by commercial marine species may become harmful to men and other animals and it is a matter of great concern today. Marine mussels are well known for their ability to accumulate pollutants from their environment and have become the subject of various monitoring programmes of the 'mussel watch' type (Goldberg *et al* 1978; Davies and Pirie 1980).

The uptake system in bivalve molluscs act as if they have first order kinetics, so that the rate at which the metal can enter the organism is proportional to the level in the ambient seawater (Phillips 1977; Lakshmanan and Nambisan 1979; Martincic *et al* 1984; Simkiss and Mason 1984; Amiard *et al* 1986; King and Davies 1987). Once the metal has entered the cells they undoubtedly become bound to a variety of ligands and it is the metabolism of these complexes that determines the subsequent fate of the metal and the final body load (Simkiss and Mason 1984). Based upon the rate of loss of a metal, its biological halflife can be determined. The biological halflife may serve as a warning about the persistence and potential for cumulative biological effects of a chemical (Buikema *et al* 1982).

Mercury is probably the most important pollutant both with regards to its effects on marine organisms and its potential hazard to man, and its compounds are included in the 'black list' of all the international conventions (Bryan 1984).

The green mussel *Perna viridis* is having wide spread distribution all along both east and west coasts of India. This is an ecologically and economically important tropical species with outstanding potential as sentinel organism (Krishnakumar 1987). Compared to temperate area mussels, little information is available on the

accumulation, distribution and depuration of mercury in *P. viridis* (Lakshmanan and Nambisan 1979). Our toxicity studies have shown that mercury is highly toxic to *P. viridis* (Krishnakumar *et al* 1987a,b). In this paper an attempt has been made to study the accumulation, distribution, depuration and biological half-lives of mercury in the different body parts of *P. viridis*. Although mussels can obtain mercury from both seawater and food, only accumulation from seawater is considered here.

2. Materials and methods

Adult specimens (35–45 mm) were collected from an unpolluted coastal area, which is remote from human activity, near Narakkal (Cochin) in March 1985. Animals were cleaned and acclimated in the laboratory for 4 days under optimal conditions (salinity $35 \pm 0.5 \times 10^{-3}$; temperature $29 \pm 0.5^\circ\text{C}$; pH 7.9 ± 0.1 and dissolved oxygen $> 4 \text{ ml l}^{-1}$). Mussels were fed with *Synechocystis* sp. and feeding was stopped during and 2 days before the accumulation study.

2.1 Accumulation study

Two exposure concentrations of 37.5 and $75 \mu\text{g l}^{-1}$ were selected after the acute toxicity (96 h LC_{50} for HgCl_2 ; $155 \mu\text{g l}^{-1}$) experiments (Krishnakumar *et al* 1987b). Twenty mussels each were placed in two separate plastic tubs, each containing 15 l of seawater for each concentration. Control and treatment tubs were kept in duplicate. Optimal conditions were maintained throughout the experiment (salinity $35 \pm 0.5 \times 10^{-3}$; temperature $29 \pm 0.5^\circ\text{C}$; pH 7.8 ± 0.1 and dissolved oxygen $> 4 \text{ ml l}^{-1}$). The test medium was changed every day and 6–8 animals were removed from both the control and treatment tubs at 1, 2, 3 and 4 days of exposure for mercury analysis. Mercury concentration in the treatment tubs were determined daily and the mean concentrations were 37.51 and $75.05 \mu\text{g l}^{-1}$ for 37.5 and $75 \mu\text{g l}^{-1}$ treatments, respectively. Seawater in the control tubs contained a mean mercury concentration of $0.026 \mu\text{g l}^{-1}$. Before the experiment few mussels were sacrificed to find out their background mercury concentrations (0 day).

2.2 Depuration study

After 4 days, mussels from the accumulation study were gently rinsed with seawater and transferred to a seawater flow-through system for depuration study. Filtered (Whatman 1) and aerated seawater is allowed to flow through the system at a rate of 150 ml min^{-1} (salinity $35 \pm 0.5 \times 10^{-3}$; temperature $28.5 \pm 0.5^\circ\text{C}$; pH 7.9 ± 0.1 and dissolved oxygen $> 4 \text{ ml l}^{-1}$). *Synechocystis* sp. cultured in metal free medium was added to the system and maintained at a concentration of $3 \times 10^3 \text{ cells ml}^{-1}$. Mussels were sampled on every 5, 10, 15, 20 and 25 days of the depuration study.

2.3 Mercury analysis

Mussels were opened and soft tissues were dissected out using clean stainless steel instruments into gills, mantle, adductor muscle + foot and the remaining body parts

as viscera. Animals (6–8) were dissected out to pool the different body parts. Soft tissues were gently washed with distilled water, blotted dry, weighed and digested using H_2SO_4 and HNO_3 mixture in Bethge's apparatus following the methods of BITC (1976). Seawater mercury levels were estimated following the method of Gardner and Riley (1974). All the analyses were done in triplicate. Total mercury was analysed by using cold vapour atomic absorption technique in a mercury analyser (ECIL, MA-77).

2.4 Data analysis

To test the linearity of mercury uptake, regression analysis was carried out using microcomputer. The linearity between the exposure period and mercury concentration in the tissue can be represented by the equation:

$$Y_t = a + bt,$$

where Y_t is the mercury concentration in the tissue at time t , b the slope of the line which is equal to rate of uptake of mercury and a the natural mercury concentration in the tissue. The bioconcentration factor (BCF) is calculated using the formula:

$$\text{BCF} = \frac{4 \text{ day tissue concentration} - 0 \text{ day tissue concentration}}{\text{Mercury concentration in the seawater}}.$$

Mercury concentration in different body parts after 4 days of exposure period was taken as the initial metal concentration (0 day) for the depuration study. Percentage of the initial concentration of mercury with the progress of depuration time was calculated. Least-square line was fitted through the semilogarithmic plots of the points. The rate of loss of mercury from the body parts of mussels is characterized by the equation:

$$\text{Log } Y = a - bx,$$

where x is the time in days, Y the percent initial concentration of mercury, a the Y intercept and b the slope of the least-square line. The biological halflives ($\text{TB}/2$) were determined by substituting the slope of the least-square line (b) in the equation (Renfro 1973)

$$\text{TB}/2 = \frac{\text{Log } 2}{b}.$$

Average $\text{TB}/2$ was calculated for different body parts from two different experiments.

3. Results

3.1 Accumulation

Significant linear mercury accumulation was found in all the body parts of mussels with progress of exposure time (figure 1). The rate of accumulation in the gills were

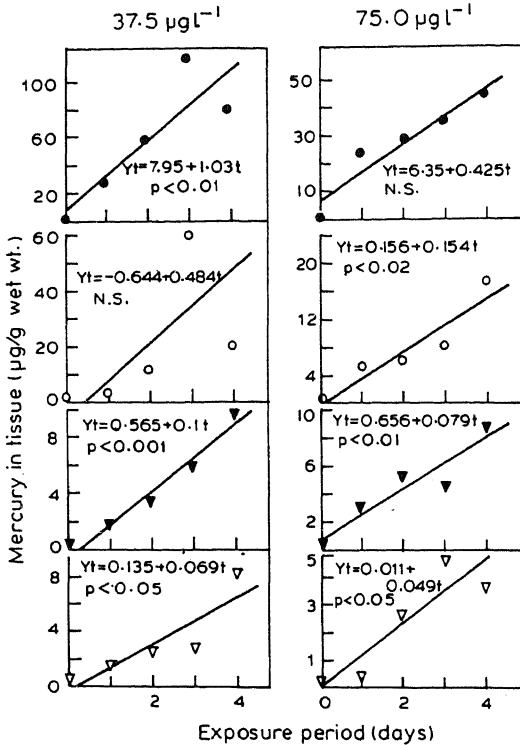


Figure 1. Accumulation of mercury in gills (●), viscera (○), mantle (▼) and muscle+foot (▽) of *P. viridis* exposed to 37.5 and $75 \mu\text{g l}^{-1}$ of HgCl_2 .

maximum, followed by viscera, mantle and muscle+foot. However, the rate of accumulation showed inverse relationship with metal concentration in all the body parts (table 1). BCF was found to be maximum in gills, followed by viscera, mantle and adductor muscle+foot. In all the body parts BCF decreased with increase in concentration of the exposure medium (table 1).

3.2 Distribution

Mussels exposed to $75 \mu\text{g l}^{-1}$ showed frequent valve closure after second day of the exposure. Gills of mussels exposed to 37.5 and $75 \mu\text{g l}^{-1}$ (will be referred hereafter in the text as the 37.5- and 75-group respectively) attained the maximum concentration of 79.92 and $45.48 \mu\text{g g}^{-1}$ wet weight respectively. In both experiments, tissue mercury levels followed the order, gills > viscera > mantle > muscle+foot.

3.3 Depuration

Mercury concentration in the different body parts of mussels after 25 days of depuration study is shown in table 1. The linearity of mercury loss and $\text{TB}/2$ in the

Table 1. Tissue concentration ($\mu\text{g g}^{-1}$ wet wt.), rate of accumulation, BCF and TB/2 of mercury in different body parts of *P. viridis*.

	Gills		Viscera		Mantle		Muscle + foot	
	a	b	a	b	a	b	a	b
Background (0 day)	3.319		0.320		0.126		0.309	
After 4 days of accumulation	79.22	45.48	20.10	17.57	9.92	8.97	8.34	3.72
Rate of accumulation	1.03	0.425	0.485	0.154	0.10	0.07	0.069	0.049
BCF	2177	604	531	227	262	117	207	48
After 25 days of depuration	40.76	25.51	16.22	12.28	4.17	4.38	4.73	2.66
TB/2(days)	31.68	21.5	81.36	64.05	19.94	24.48	38.11	47.78
Mean TB/2 (days)	26.37		72.71		22.11		42.95	

a, $37.5 \mu\text{g l}^{-1}$; b, $75 \mu\text{g l}^{-1}$.

different body parts are shown in figure 2. After 25 days 37.5-group showed only 19% reduction in viscera. Mantle showed maximum reduction of 53%. In 75-group maximum reduction (51%) attained in mantle, while in muscle and viscera mercury reduction was slow (30%).

In 37.5-group, viscera showed longest TB/2 (81.36 days), while mantle showed shortest TB/2 (19.94 days). In 75-group also viscera showed longest TB/2 (64.05 days), while gills showed shortest TB/2 (21.5 days). The order of mean TB/2 in the different body parts are: viscera > muscle + foot > gills > mantle.

4. Discussion

The mercury accumulation pattern observed in different body parts was found to be different from each other (figure 1). This may be due to the differences in the physiological functions these tissues perform (Eganhouse and Young 1978). In the present study maximum mercury accumulation was observed in the gills followed by viscera, while minimum in the mantle and muscle + foot. Similarly higher accumulation of inorganic mercury was reported in gills, kidney and digestive glands and lower in the adductor muscle and foot of *M. edulis* (King and Davies 1987). High mercury concentrations were reported in the gills of bivalves by several authors (Unlu *et al* 1972; Dillon and Neff 1978; Lakshmanan and Nambisan 1979; Denton and Burdon-Jones 1981). These type of high metal uptake in the gills may be due to their functional activity and position relative to incoming ambient water (Dillon and Neff 1978). Moreover absorption of the metal ions by the mucous sheets may also add to the greater concentration in the gill tissues as reported by Smith *et al* (1975).

After 4 days of exposure, mercury distribution in the different body parts of

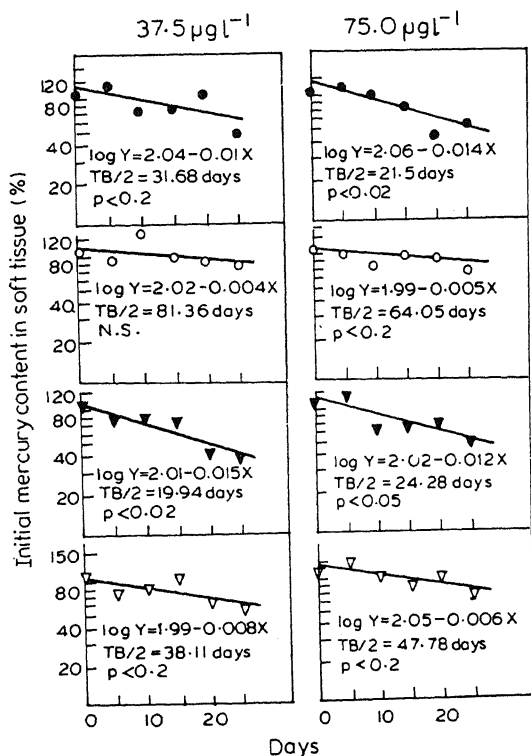


Figure 2. Depuration of mercury from gills (●), viscera (○), mantle (▼) and muscle+foot (▽) of *P. viridis*.

P. viridis indicates that the ability of the animal to accumulate mercury vary from organ to organ as reported in *Mytilus edulis* (Amiard *et al* 1986). In the present study mussels showed a time integrated linear accumulation of mercury (figure 1). Smith *et al* (1975) reported an increase in rate of accumulation of mercury by freshwater clams, with increase in concentration in the medium. However, the reverse was observed in the present study. This may be due to the higher concentration of mercury used in the exposure study ($75 \mu\text{g l}^{-1}$). Valve closure and reduction in filtration rate was observed in bivalves exposed to higher concentration of metals (Akberali and Trueman 1985). Mercury concentration of $74 \mu\text{g l}^{-1}$ was found to be inhibiting 50% of the filtration rate (EC_{50}) in *P. viridis* (Krishnakumar 1987).

Results of the depuration study clearly indicate that the ability of different body parts to eliminate mercury vary from each other. Comparatively rapid mercury loss was found in the external tissues like gills and mantle (figure 2). Most probably the excretion of mercury might have occurred across the body surface of gills and mantle, since these tissues are always in direct contact with the ambient medium. Similar rapid decline of mercury was noticed in gills together with an increase in foot and muscle of bivalves (Cunningham and Tripp 1975; Smith *et al* 1975; Fowler *et al* 1978; Denton and Burdon-Jones 1981).

Comparatively less mercury was lost from viscera and muscle+foot of mussels in

the present study. Similarly, high amount of mercury was found to be retained in the visceral organs of *M. galloprovincialis* and *Tapes decussatus* (Miettinen *et al* 1972). In *Rangia cuneata* large amount of mercury was retained in viscera after 8 days of depuration (Dillon and Neff 1978). These types of high mercury retention in viscera may be due to slower release of metal or due to a net internal flow towards viscera as noticed by Dillon and Neff (1978).

The dosage, duration of exposure to a specific metal, duration of depuration and physiological conditions of the animals may affect the TB/2 of the heavy metals (Unlu *et al* 1972; Cunningham and Tripp 1973). Metallothioneins were found to be playing a fundamental role in accumulation and elimination of heavy metals in mussels (Roesijadi 1982; Viarengo *et al* 1985). They suggested that the different TB/2 of metals are related to the different capacity of cells to eliminate the metals bound to thioneins or metallothioneins themselves. The slow depuration of mercury from viscera and muscles could be related to the chemical and physical difficulty in removing mercury or mercury containing substances (like metalloprotein) to the exterior (Dillon and Neff 1978; Viarengo 1985).

Cunningham and Tripp (1975) have recognised following categories of heavy metal release: (i) increase in TB/2 with increase in body burden of heavy metals, (ii) stable TB/2, when an equilibrium is maintained with a proportionate increase in the rate of heavy metal loss as its body burden increase, and (iii) decrease in TB/2 with increase in body burden. In the present study, release of mercury from the viscera apparently followed the first category of metal release and from the gills followed the third category.

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Biology of *Apanteles machaeralis* Wilkinson (Hymenoptera: Braconidae) a parasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae)

CLEMENT PETER and B V DAVID

Fredrick Institute of Plant Protection and Toxicology, Padappai 601 301, India

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Abstract. Studies were conducted on the endoparasite *Apanteles machaeralis* Wilkinson (Hymenoptera: Braconidae) to determine its biology when *Diaphania indica* (Lepidoptera: Pyralidae) is its host. Eggs were deposited in host larvae and increased greatly in size before hatching. The first instar larva was very active with functional mandibles, whereas the second stage larva was quiescent. The third instar larva spins its cocoon outside the body of the dead host. Mean development time from egg to adult was 12.63 days at $29.25 \pm 1.82^\circ\text{C}$ and 59–66% RH. There is no preoviposition period. The sex ratio was 1:1.22 (males/females). Mean adult longevity was not significantly different for males (9.31 days) and females (11.68 days).

Keywords. *Apanteles machaeralis*; biological control; biology.

1. Introduction

The pumpkin caterpillar, *Diaphania indica* (Saunders) has been reported from several parts of India and other regions of the world causing damage to various cucurbitaceous plants. Patel and Kulkarny (1956) have conducted detailed studies on the biology of this insect on *Coccinia grandis* (L.) Voight in Gujarat. However literature on the natural enemies of *D. indica* is very meagre. During the course of the studies on the natural biological control of this insect pest a solitary endoparasite, *Apanteles machaeralis* Wilkinson was reared as a major parasite of *D. indica* from Padappai. Previously, Bhatnagar (1948) reported *A. machaeralis* as a parasite of *D. indica* from Bihar. The present study was carried out since there is no information available on the biology of *A. machaeralis* as a parasite of *D. indica*.

2. Materials and methods

2.1 Rearing method and maintenance of stock culture

The adults which emerged from field collected parasite cocoons were fed with 20% honey solution. After 24 h the females were separated out. Twenty-five first instar larvae were released on *Coccinia* leaves inserted into a glass vial (6×1.5 cm) placed inside a plastic jar (12×10 cm) and 3 mated females were released into each jar. The larvae were exposed to the parasite for 12 h. At the end of this period the parasites were removed from the jar and the larvae allowed to feed on the leaves. The leaves were changed periodically until the parasite larvae completed their development inside the host larvae and the parasite cocoons were formed. These cocoons were

collected from the rearing jars and placed in specimen tubes until the adults emerged. These adults were fed with 20% honey solution and used again for further rearing.

The laboratory culture of this parasite was frequently rejuvenated by mixing with field collected material.

2.2 Life history and morphology of immature stages

To study the life history of the parasite one-day old females were selected from the stock culture. They were placed in specimen tubes measuring 15×2 cm. A first instar larva was taken on a fine camel hair brush and placed close to the female in the tube. After oviposition by the parasite the larva was placed in a plastic jar with *Coccinia* leaves. This procedure was repeated until the female refused to show any interest in the larva offered to it. Larvae were exposed to 10 females daily and those exposed to the same female on a particular day were placed together in plastic jar and the date recorded on it.

To determine the incubation period the larvae were dissected at periodic intervals of 6 h and the development of the eggs was followed until hatching was observed. When the egg was fully developed, observation period was narrowed to 3 h. Parasite eggs were removed from the host larvae at periodic intervals and measured with a calibrated ocular micrometer using a compound microscope.

To establish the larval period the parasitized host larvae were dissected at periodic intervals of 12 h using a Carl Zeiss Zoom Citoval-2-stereo-microscope. The parasite larvae were measured with a calibrated ocular micrometer and drawn using a camera lucida attached to a Carl Zeiss Laboval 4 compound microscope. Magnification used for drawing the larval stages ranged from X32 to X640. This arrangement for measuring and drawing of the various stages was made use for all the biology studies carried out in the present investigation. The drawings formed only an outline of the various immature stages and do not represent the accurate morphological features as indicated in the text. The constant movement of the live specimens rendered the drawing process very difficult.

The larval stages were determined by studying the shape and size of mandibles at different stages. To determine the shape and size of the larval mandibles the larvae were boiled in a 10% KOH solution for 45 s for clearing but not completely removing the host tissues. After being washed in distilled water they were mounted in Hoyer's medium on microscope slides. The head, capsules and mandibles were also measured.

The cocoons were dissected using a microscissor at 12 h intervals to record the prepupal and pupal periods. Both stages were measured.

In order to determine the fecundity, 25 host larvae were exposed for 24 h to a single mated female in a plastic jar. The larvae were reared on *Coccinia* leaves. Three replications were maintained. After 24 h the host larvae were dissected and the number of eggs present in each larva was counted. This procedure was repeated until all females died. From this data the total number of eggs laid by each female was estimated.

Field collected cocoons were observed for emergence of adults which were sexed to assess the sex-ratio.

To study the host stage preference for oviposition, larvae of *D. indica* of the following age groups were selected: 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9 and 9-10-day old larvae. Twenty-five larvae belonging to each age group were released on *Coccinia* leaves and placed in a jar with wire mesh fitted lid. A single mated female was released into each jar. Three replications were maintained for each stage exposed. The number of cocoons formed for each stage was recorded at the completion of the larval development of the parasite.

The pre-oviposition and oviposition periods were determined by exposing *D. indica* larvae to the parasite females at regular intervals of 24 h beginning with the day of emergence. The larvae were dissected after each exposure and those having parasite eggs were determined. Host larvae were exposed continuously until the female died. Five replications were maintained for this study.

The adult specimens were initially preserved in 70% alcohol. These specimens were then mounted on slides using the following methodology. The specimen was treated in 10% KOH for 10-12 h for clearing the tissues and then transferred to glacial acetic acid to dissolve the organic matter, if any. It was passed through carboxylol. The processed specimens were dissected in clove oil and the different parts were mounted on slides in DPX mounting medium. Slide mounts of complete specimen were also prepared. The various parts of the adult were measured.

3. Results and discussion

3.1 Immature stages

3.1a Egg: The egg immediately after deposition is elongate with a long thin pedicel. The chorion is thin, transparent and devoid of any sculpturing. The egg develops rapidly after deposition and changes greatly in size and shape. When freshly laid it measures 0.043 mm long from tip to the end of the pedicel and 0.056 mm wide. After 6 h the length increases to 0.48 mm and in width to 0.1 mm. At the end of 12 h after deposition, the egg measures 0.532 mm long and 0.102 mm wide at the broad end. After 20 h, the fully developed embryo is clearly seen inside the chorion. The full grown egg just before hatching measures 0.62 mm long and 0.12 mm wide at the broad end (figure 1A-D). Thus within 24 h after oviposition there is an increase of 1.45 times in size compared to the freshly laid egg. The width of the egg also increases to 2.4 times the original size. This increase is more pronounced in the transverse direction.

The general shape and size of the egg of *A. machaeralis* is similar to several other species of solitary *Apanteles* studied. The rapid increase in the size of the egg after oviposition has been reported for other species too. In *A. solitarius* Ratzeburg an increase of 1.96 times in length and 1.5 times in width was reported at the time of hatching (Parker 1935). A 4-fold increase in width and 1.25 times increase in length has been reported for *A. angaleti* Muesebeck (Narayanan *et al* 1956). Allen (1958) recorded an increase of 1.58 times in length and 2.88 times in width for *A. medicaginis* Muesebeck. Cardona and Oatman (1971) reported an increase of 1.37 times in length and 2.4 times in width for *A. dignus* Muesebeck. In certain Euphorinae and Meteroinae the increase is even greater (Balduf 1926).

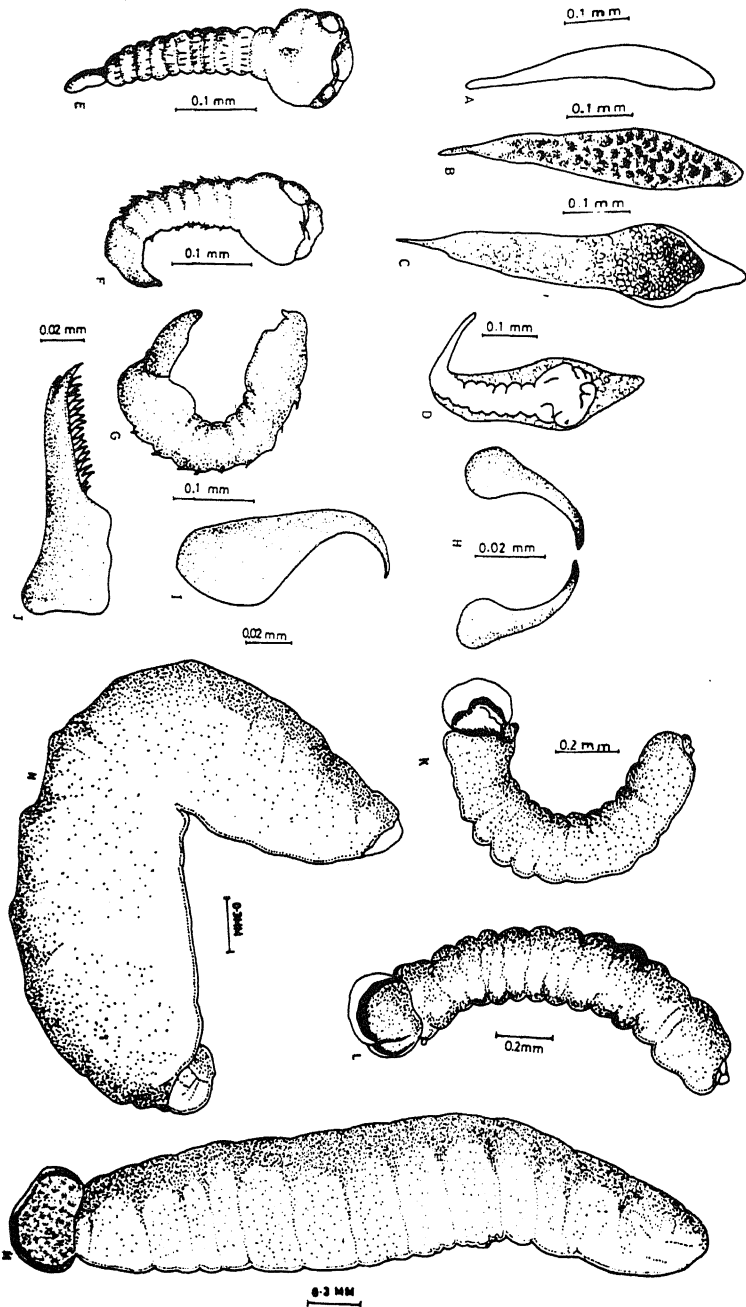


Figure 1. Developmental stages of *A. machaeralis*. A–D, Egg; E–G, first instar larva; H, first instar mandible; I, second instar mandible; J, third instar mandible; K, second instar larva; L–N, third instar larva.

3.1b *Larva*: (i) *First instar larva*: The measurements made relating to various developmental stages of *A. machaeralis* are shown in table 1. The freshly hatched larva has a broad head about twice the width of the body. The head measures

Table 1. Mean size and duration of immature *A. machaeralis* reared at $29.25 \pm 1.82^\circ\text{C}$.

Stage	n	Length (mm)	Width (mm)	Duration (day)
		$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$	
Egg	25	0.62 ± 0.14	0.12 ± 0.03	18–24 h
1st instar	20	0.80 ± 0.12	0.08 ± 0.01	3–4
2nd instar	12	1.31 ± 0.19	0.29 ± 0.08	1–2
3rd instar	12	3.11 ± 1.11	0.82 ± 0.10	2–3
Prepupa	12	2.88 ± 1.21	0.78 ± 0.14	1–2
Pupa	20	2.78 ± 1.08	0.89 ± 0.26	4–7

0.12 mm in width while the width of the body is 0.06 mm. At the time of hatching it measures 0.33 mm in length and 0.06 mm wide. The larva at this stage is distinctly segmented (figure 1E–G). The body is made up of 3 thoracic and 7 abdominal segments. These segments have a single row of transverse sharp translucent dorsal spines. The caudal horn is prominent in the freshly hatched larva but it shrinks gradually as the larva develops while the anal vesicle increases in size. The caudal horn measures 0.08 mm on the day of hatching decreases to 0.04 mm on the third day and is completely absent at the end of first instar. During this period, the anal vesicle enlarges from 0.11 mm in width on the second day to 0.48 mm at the end of first instar. Even the dorsal spines which are prominent at the beginning slowly reduce in size and disappear as the larva develops.

The characteristic feature of this instar is the sickle shaped mandibles which are well fitted for tearing (figure 1H). The mean length of the mandibles measures 0.46 mm from the tip of the spine to the point of the basal process and 0.019 mm in width at the widest point. The mandibles are chitinized throughout, but more heavily so at the tips and form a good character for distinguishing this instar from the subsequent instars. The first instar larva is very active and its mandibles are constantly in motion. It is probably through the use of these mandibles that any other species or members of the same species encountered within the host are destroyed.

(ii) *Second instar larva*: The second instar larva differs considerably from the first instar larva although the change is gradual. In fact, the transition is very difficult to detect. The body tapers from the large anal vesicle toward the head (figure 1K). The head has no apparent sclerotization and the mouth parts are not visible. The mandibles, 0.09 mm in length are colourless and difficult to detect. The base of the mandible is very broad and tapers to the blade (figure 1I). The body is distinctly segmented with 10 abdominal and 3 thoracic segments. Abdominal spines are lacking. The body is opaque and creamy white. The anal vesicle, 0.259 mm in width, is a greatly enlarged and prominent structure while the caudal horn is absent.

(iii) *Third instar larva*: The change into the third instar is again difficult to differentiate from the second instar because of its similarity in appearance. The shape of the mandibles is a characteristic feature of this instar. The mandibles are strongly chitinized with series of 17 saw-like teeth on the inner edge (figure 1J). These mandibles are used by the parasite larva to cut its way out of the host.

The third instar larva is creamy white and opaque. The body tapers anteriorly and is robust. The head capsule is well sclerotized with mean width of 0.559 mm (range 0.481–0.612). The body consists of the head and 13 well developed segments.

3.3 Adult

3.3a *Description*: Female: 3.03 mm long. Body black in colour. Head 0.55 mm long and 0.61 mm wide; antennae 2.99 mm long, 19 segmented; eyes black, 0.27 mm long and 0.13 mm wide. Thorax 1.03 mm long and 0.80 mm wide. Wings hyaline and transparent, veins pale in colour, fore wings 2.35 mm long and 0.55 mm wide; hind wing 1.18 mm long and 0.23 mm wide. Legs black to yellowish brown; foreleg femur black at the base, the rest of the femur, tibia and tarsomeres yellowish brown; middle leg femur more or less completely black, tibia and tarsomeres yellowish brown as in foreleg; hind leg femur completely black, tibia light yellowish brown to black; tarsomeres yellowish brown. Abdomen black, 1.37 mm long; ovipositor 1.32 mm long and black in colour.

Male: Identical to female except slightly smaller in size, body length 2.49 mm. Antennae 3.03 mm long and longer than that of female.

3.3b *Mating*: Mating occurs soon after emergence. The males actively follow the females with vibrating wings. If the female is receptive it stops and spreads its wings and the male approaches the female from behind. The male, after mounting, curves its abdomen down and inserts its aedeagus into the genital aperture of the female. Copulation lasts 10–20 s. After mating, the female move away and begins to groom itself while the male moves about wildly in search of other females. The position taken during mating is common to other species of *Apanteles* and the act is completed in less than a minute.

3.3c *Preoviposition period*: The experiment conducted to determine the preoviposition period of *A. machaeralis* females revealed that egg laying commenced on the day of emergence irrespective of mating. The adults of *A. melanoscelus* (Crossman 1922), *A. solitarius* (Parker 1935) and *A. dignus* (Cardona and Oatman 1971) were reported to be ready for oviposition within a few hours after they issue from the cocoons.

3.3d *Oviposition behaviour*: Oviposition takes about a second and the female inserts its egg into the thorax close to the head. The host larva thrashes out violently when the female thrusts its ovipositor into the body. If the same larva is exposed again to the female another egg is laid and in some cases up to 3 eggs were deposited in one larva but only one survives after hatching. Oviposition is similar to other species of *Apanteles* and is completed very rapidly. It was reported to take one second for *A. melanoscelus* (Crossman 1922), 3–12 s in *A. angaleti* (Narayanan *et al* 1956), 1 s in *A. medicaginis* (Allen 1958) and 2–3 s in *A. dignus* (Cardona and Oatman 1971). The site of oviposition varied in some species of *Apanteles*. While *A. melanoscelus* favours the posterior half of the larva (Crossman 1922) the female of *A. solitarius* oviposits in any part of the host larva (Parker 1935). In the solitary species of *Apanteles* only one egg is generally laid with each thrust. In *A. medicaginis* the number of eggs ranges from 1–6 per host (Allen 1958) but only one larva completes development in the solitary forms of *Apanteles* sp.

3.3e *Oviposition period*: The average oviposition period of *A. machaeralis* was

8.60 days (range 3–11 days). In most cases oviposition continued as long as the female was mobile. However, the rate of egg laying gradually reduced with age. In *A. dignus* the average oviposition period was reported to be 6.7 days which was almost the same as average female longevity (Cardona and Oatman 1971).

3.3f *Host stage preference for oviposition:* Maximum parasitism occurs in 1–2-day old larvae (72%) and 2–3-day old larvae (76%). Larvae up to 5 days old were also attacked but to a lesser extent (<10%). It was determined from the present study, that first instar larvae of *D. indica* are preferred for oviposition by *A. machaeralis*. Certain variations in the host age preference for oviposition by other solitary forms of *Apanteles* spp. have been recorded in literature. Crossman (1922) reported that third instar larvae are preferred by *A. melanoscelus*. In *A. medicaginis* the first instar larva is generally selected for oviposition (Allen 1958) and in *A. dignus* 2–3-day old larvae are the most suitable ages for parasitization (Cardona and Oatman 1971).

3.3g *Sex ratio:* Parasite cocoons obtained from field collected larvae were held until adults emerged. The sex ratio of adults from 140 cocoons was 1.22:1 in favour of females. *A. machaeralis* is an arrhenotokous species. Unfertilised females deposit eggs that develop into males and the progeny of mated females contains both sexes. This type of reproduction which is common in most *Apanteles* is described in detail by Allen and Smith (1958). In *A. angaleti* the male: female sex ratio was reported to be 1:2 (Narayanan *et al* 1956), while for *A. dignus* the sex-ratio was recorded to be 1.47:1 in favour of males (Cardona and Oatman 1971).

3.3h *Fecundity:* The fecundity of mated females of *A. machaeralis* ranges from 80–126 eggs per female with a mean of 118.56 eggs under insectary conditions. In the field the oviposition ratio may be higher. There was considerable variation in the fecundity of other solitary *Apanteles* spp. Crossman (1922) reported that females of *A. melanoscelus* were capable of laying about 1000 eggs under natural conditions. However in the laboratory the fecundity was estimated to be 535 eggs per female. The reproductive capacity of *A. solitarius* ranged from 311–516 with an average of 402 (Parker 1935). In *A. angaleti* the average fecundity was reported to range from 61.83–86.33 per female (Narayanan *et al* 1956). Cardona and Oatman (1971) reported that in *A. dignus* the total production of progeny by mated females ranged from 126–182.

3.3i *Longevity:* In the absence of food the mean longevity of females was 1.63 days and of males 1.48 days. Both sexes lived longer when provided with 20% honey solution with the mean longevity being 11.68 days for females and 8.31 days for males. *A. melanoscelus* was reported to live for 30–32 days (Crossman 1922) with no difference in the longevity of the two sexes. Parker (1933) reported that *A. solitarius* lived for an average of 3–4 weeks and the females lived longer than males. *A. angaleti* was reported to live for 2–8 days (Narayanan *et al* 1956). Cardona and Oatman (1971) observed that in *A. dignus*, males lived longer (13.6 days) than females (10.2 days) and without food or water the mean longevity was 1.2 days for males and 1.1 days for females.

3.4 Hyperparasite of *A. machaeralis*

During this study only one hyperparasite emerged from the cocoon of *A. machaeralis*; it was subsequently identified as *Elasmus hyblaeae* Ferriere. This is the first record of *E. hyblaeae* as a hyperparasite of *D. indica* through *A. machaeralis*.

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Histopathological observations on the digestive gland of *Lymnaea auricularia* infected with larval trematodes

S L CHOUBISA

PG Department of Zoology, S B P Government College, Dungarpur 314 001, India

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Abstract. Histological observations were made on the digestive gland of infected and uninfected freshwater snails, *Lymnaea auricularia*, and the varying degree of histopathogenesis of echinostome cercaria, *Cercaria itoi*, as well as strigeoid metacercaria, *Tetracotyle lymnaei*, along with *Cercaria itoi* (double infection) in the digestive gland have been observed. Basically 3 types of lesions, L_1 , L_2 and L_3 were found and were associated with the types and size of trematode larvae as well as with the degree of parasitemia. In the early stage of larval development or in mild infection, type I lesions (L_1) were commonly observed in which degenerative changes were confined to individual digestive gland tubules, and neighbouring tubules mostly remained healthy or unaffected. The type II lesions (L_2) were associated with severe infection and were less common than L_1 lesions and large areas of parasitised digestive gland tubules were revealed to be necrotic. Type III lesions (L_3) were also associated with severe infection in which digestive gland tubules appeared as rudimentary tubules located between trematode rediae. In the present study, in addition to a description of the different types of lesions, the various alterations of cellular structures of the digestive gland of *Lymnaea auricularia* due to single or double infections (*Cercaria itoi* + *Tetracotyle lymnaei*) are also discussed.

Keywords. Histopathology; digestive gland; *Lymnaea auricularia*; *Cercaria itoi*; *Tetracotyle lymnaei* (metacercaria); lesions.

1. Introduction

Infection by most trematodes, adults as well as their larval stages cause considerable damage to the host tissues. Histopathological changes in molluscs caused by larval trematode infections have been described by many (James 1965; Patnaik and Ray 1966; Moore and Halton 1973; Mohandas 1974, 1977; Yoshino 1976; Sommerville 1978; Bertman 1980; Choubisa 1988), and reviewed by Cheng and Snyder (1962), Wright (1966) and Erasmus (1972). However, none of these workers has attempted to give a comparative histopathological account of the digestive gland associated with single and double infection. Although, many have reported instances of double and multiple infections (Mohandas 1971; Choubisa and Sharma 1983a, 1986) in snail host; cases of two different developmental stages (cercaria + metacercaria) of different species of trematodes occurring in the same snail species are rarely reported. In the present study this point has been highlighted along with histopathogenesis of the echinostome cercaria, *Cercaria itoi* in the digestive gland of *Lymnaea auricularia*.

2. Materials and methods

During a survey of freshwater larval trematodes and their snail hosts in southern Rajasthan (Choubisa and Sharma 1983a, 1986) specimens of the snail species,

L. auricularia, were collected and examined for larval trematode infection. The methods of rearing the snails, and collection and identification of the larval trematodes were the same as described earlier (Choubisa and Sharma 1986). The infected digestive glands either with echinostome cercaria, *C. itoi*, and its redia or strigeoid metacercaria, *Tetracotyle lymnaei* along with *C. itoi* and its redia (double infection) were fixed in 10% buffered formal saline or Bouin's fixative. Tissue blocks were prepared and sections cut at 7 μ m on a rotary microtome. Sections were stained with haematoxylin and eosin.

3. Results

Of the 250 *L. auricularia* examined during winter (December to January) 32 (12.8%) were infected with echinostome cercaria, *C. itoi*, and 5(2%) with this cercarial species along with the metacercaria, *T. lymnaei*.

3.1 Morphology and histology of uninfected digestive gland

The healthy digestive gland of *L. auricularia* was found to be grey in colour and occupied the maximum part of the whole of the visceral mass which was enclosed by a thin membrane, the tunica propria.

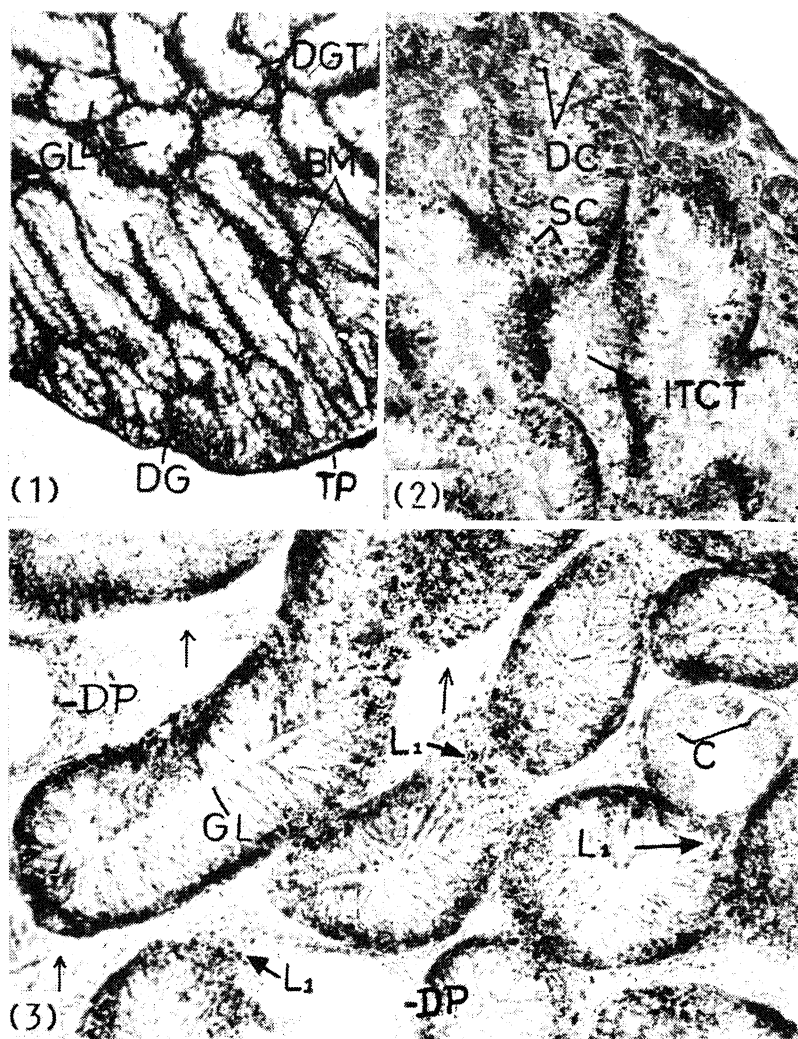
The basic histological feature was the mass of digestive gland tubules embedded in their stroma of fine connectives (figures 1, 2). The tubule consists of a single layer of epithelial cells on a prominent basement membrane. The epithelial cells are differentiated into 3 types on the basis of their morphology. The digestive cells (absorptive cells) are ciliated columnar with basal nuclei and numerous globules. These were observed most frequently. The second type of cells were the secretory cells, oval to triangular shaped with base abutting the basement membrane and the apex disposed towards the digestive lumen. Their nuclei were comparatively large and were more numerous in some digestive tubules. The third type of cells, undifferentiated cells or thin cells, were seen only in the intestinal component of the digestive epithelium.

The space between digestive gland tubules was filled with a fine and unbroken network of intertubular connective tissues (figures 1, 2). The intraglandular space or gut lumina in healthy digestive glands was found to be very reduced or compact. The unbroken intertubular connective tissues and compact gut lumina reflect healthy condition of the digestive gland.

3.2 Morphology and histology of parasitized digestive gland

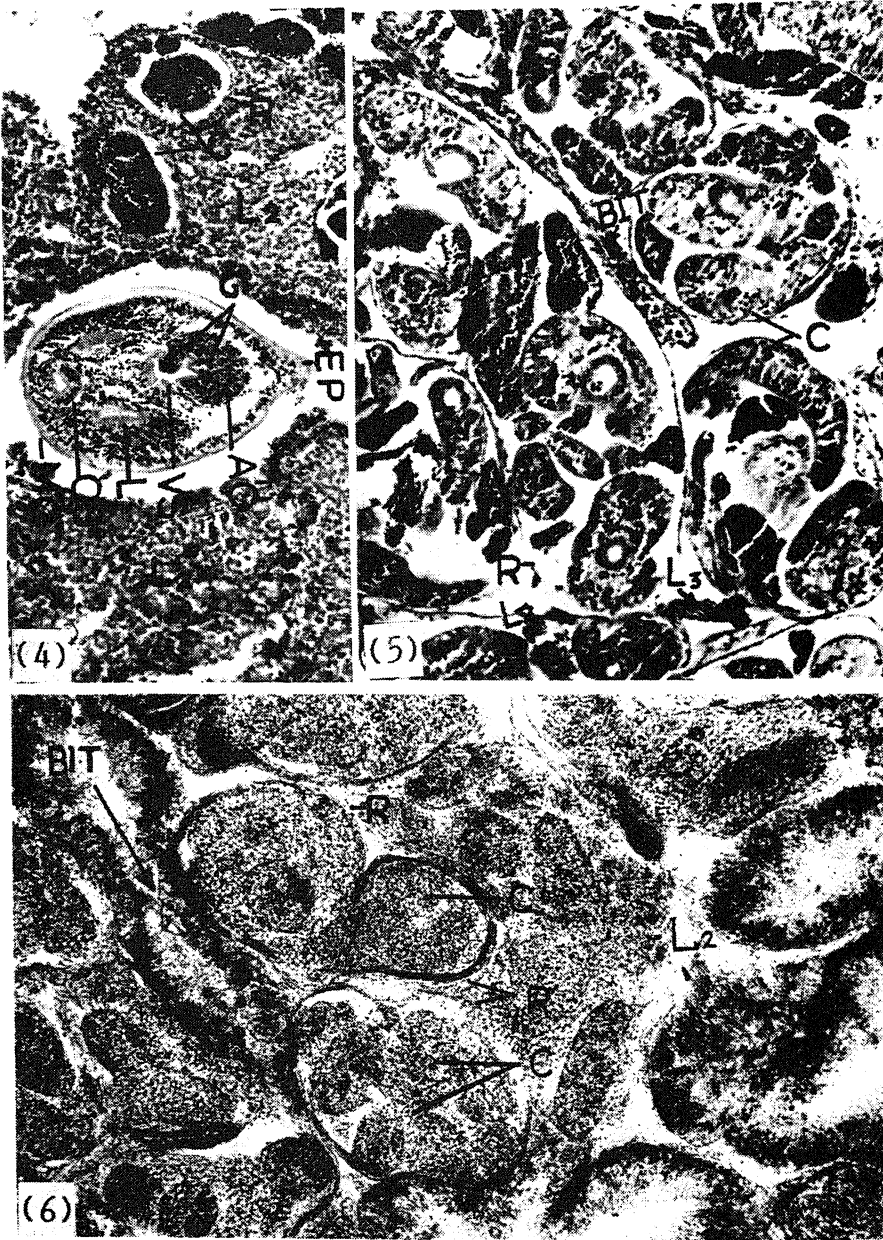
Infected digestive glands of *L. auricularia* either with single (*C. itoi*) or double (*C. itoi* + *T. lymnaei*) infections appeared to white in colour, swollen and in a loose mass. The tunica propria was also seen under pressure.

In most cases large areas of intertubular connective tissues were occupied by parthenitae. Tubules were reduced in diameter, number and irregularly shaped but their lumina were found to be enlarged (figure 3). Similarly, the intertubular spaces were also found to be enlarged. In the parasitized digestive gland, either with single or double infections, 3 types of lesions could be distinguished. In the type I lesion



Figures 1-3. Histological section of healthy digestive gland of *L. auricularia* stained with haematoxylin and eosin ($\times 75$). 2. Section of healthy digestive gland enlarged ($\times 240$). 3. Section of infected digestive gland with *C. itoi* showing lesion L_1 ($\times 240$).

(L_1), degenerative changes were confined to individual tubules and neighbouring tubules were unaffected (figure 3 indicated by L_1). Such types of lesions occurred in mild infections with larval parasites. The number of globules in the cytoplasm of digestive cells was reduced, consequently the diameter of tubules became progressively smaller and the intertubular lumina progressively occluded (figure 6). The lateral walls of digestive cells as well as basement membrane appeared to be broken leading to complete loss of cell organization (figure 3). As a result of gradual advancement of infection, the height of columnar cells became reduced, and the lumina was found enlarged. The secretory cells were the last to lose their integrity and remained free within the lumina. Intertubular areas were found to be progressively enlarged and filled with transudate associated with the destruction of



Figures 4-6. 4. Section of infected digestive gland with *C. itoi* and metacercaria, *T. lymanaei* showing lesion L_2 ($\times 240$). 5. Section of very severely infected digestive gland with *C. itoi* showing lesion L_3 ($\times 240$). 6. Section of severely infected digestive gland with *C. itoi* showing blocking of tubules along with lesion L_2 ($\times 240$).

(AO, Adhesive organ; BIT, blocking of individual tubule; BM, basement membrane; C, cercaria; DC, digestive cells; DG, digestive gland; DGT, digestive gland tubules; EP, excretory pore; G, gonads; GL, gland lumen; ITCT, intratubular connective tissues; L, lappets; MC, metacercaria; OS, oral sucker; R, redia; SC, secretory cells; VS, ventral sucker).

the tubules (figure 3, indicated by everted arrows). In severe infections, cells of tubular epithelium in close apposition to redia appeared to be fragmented.

The type II lesions (L_2) associated with severe infection or greater degree of parasitemia were frequently observed in single infections (figure 6) but common in double infections (figure 4). Type L_2 in either type of infection was characterized by the development of large foci of severe acute liquefactive necrosis in a gland which otherwise manifested the features of the type L_1 lesion. In the necrotic areas, unorganized columnar digestive cells along with clusters of rediae and cercariae were found, but secretory cells remained virtually intact within affected areas. In double infections, there was complete necrosis of digestive gland but in single infection some tubules still remained intact.

The type III lesions (L_3) were observed only in the case of severe single infection (figure 5). L_3 were characterized by atrophy of digestive tubules, and tubules appeared as small mass of cells but necrosis was not found.

4. Discussion

It is clear from the present investigation that the variety of histopathological changes in snail tissues induced by larval trematodes depend upon the severity or degree of infection, size and types of larvae. The mechanical damages (L_1) of the digestive tubules appear to be the cumulative effect of larval migration, feeding and asexual reproduction, whereas autolytic necrosis (L_2) is the result of the release of proteolytic enzymes from the ruptured digestive cells/or enzymatic secretion from trematode larvae (Choubisa and Sharma 1983b; Sharma and Choubisa 1985; Choubisa 1986). In heavy infection with rediae of *C. itoi* the digestive gland tubules at various places between well developed rediae became compressed or blocked. Consequently, starvation autolysis or atrophy (L_3) has taken place. However, the root of complexity lies in the degree of parasitemia, age/sizes and types of trematode larvae.

Common pathological changes, such as blocking of digestive tubules and reduction in height of columnar cells in the form of squamous or cuboidal as well as increase in inter and intratubular spaces as observed in the present study may be due to (i) intracellular digestion at the time of starvation in *L. auricularia* causes starvation autolysis and atrophy of the gland and (ii) the reduction in the amount of storage nutrients due to increasing demands of the developing host gonads as well as developing or multiplication of larvae. The former point gets support from the work of James (1965) and Mohandas (1974) and the latter from the work of Bertman (1980). The pathological alterations in the digestive gland of the snail host of the present study can be classified basically into two, mechanical and physiological. There may be several factors involved in such pathogenesis. Both types of damages may have come from the blocking of digestive tubules by growth, multiplication and movement of trematode larvae. The severity of mechanical damage also depends upon the type and size of trematode larvae as well as their developmental stages. Mohandas (1977) and Choubisa (1988) have also discussed that radial stages produce greater mechanical as well as physiological damages than sporocysts. Rediae have mouth and locomotory organs which are responsible for a great deal of mechanical damage whereas their pharyngeal glands, and digestive system are responsible for the physiological damages. Some times redia engulfs digestive cells and also uses its hydrolase enzymes for extracellular digestion

(Sharma and Choubisa 1985). Thus both types of damages take place with radial infection and this factor must also be considered. Other contributory factors are parasitic secretions and excretions which produce toxic effects. In the present study, greater pathogenesis (both types of damages) was found in the case of double infection since excysted metacercaria, *T. lynnaei* and rediae of echinostome cercaria have well developed locomotory organs and digestive system. Double infection actually contributes twice the hydrolases than single infection in the digestive gland of *L. auricularia*.

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Amino acids, aminotransferases and proteins in the metamorphosing silkworm, *Bombyx mori* L.

S SIVA PRASAD and P MURALI MOHAN

Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India

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Abstract. The total free amino acid levels declined from the early-fourth instar to the mid-fifth instar and were elevated during the late-fifth instar in the metamorphosing silkworm, *Bombyx mori*. The activity levels of aspartate and alanine aminotransferases showed an increase in the silk gland while they decreased in the central nervous system, muscle and hemolymph. The molting period was, however, characterized by low activity levels in all the tissues studied. The total and soluble protein levels increased continuously during metamorphosis. The results are correlated with active protein synthesis and silk production in the developing *Bombyx mori*.

Keywords. *Bombyx mori*; metamorphosis; central nervous system; amino acids; aminotransferases; proteins.

1. Introduction

Species-specific variations in the ontogenic pattern of various biochemical constituents are an essential feature of insect metamorphosis. The study of Chen (1971) provided an innovative report on the role of biochemical constituents during insect metamorphosis. Biochemical studies on the silkworm have been initiated with the aim of understanding the biochemical correlates of silkworm metamorphosis and silk production. The biochemical parameter which attracted considerable attention in this regard is the silk protein fibroin. The metamorphosis of silkworm involves rapid growth of the silk gland and increased protein and fibroin synthesis. Silkworm proteins and their developmental changes have been examined (Robert and Rutt 1982; Sarangi 1985). Pursuits in this direction have led to studies on the amino acids and on the enzymes involved in protein and amino acid metabolism (Bheemeswar and Sreenivasaya 1952; Inokuchi and Yoshitake 1978; Keiji and Daillie 1978; Pant and Jaiswal 1981; Sumio *et al* 1981; Bannikov *et al* 1982; Giordana *et al* 1982; Wanger-Li and Xuting-Sen 1982; Parenti *et al* 1985). These studies were mostly performed on tissues other than the nervous system. Since the nervous system controls the overall activity of the animal it is essential to study the biochemical changes in this system during silkworm metamorphosis, which might eventually give the clue to the role of this facet in silk production. Hence in the present study some aspects of protein metabolism have been examined in the central nervous system (CNS), muscle, silk gland and hemolymph during the development of the silkworm, *Bombyx mori*.

2. Materials and methods

The present investigation was carried out on LR×NB₄D₂ (multivoltine pure Mysore×bivoltine NB₄D₂) hybrid variety of the silkworm, *B. mori*. The silkworms

were reared in large bamboo trays in the laboratory as per Krishnaswami (1986).

The fourth (lasting 5 days) and fifth (lasting 8 days) instar larvae of the silkworm were selected for these studies, as enough tissue could be obtained from them for analysis. For experimental convenience, the duration in both instars was divided into 3 stages, viz. early, middle and late. 1st, 3rd and 5th days of the fourth instar and 1st, 5th and 8th days of the fifth instar were considered as the early, middle and late stages respectively.

The levels of free amino acids, both soluble and total proteins and the activity levels of aspartate (AAT) and alanine aminotransferases (AIAT) were estimated in the CNS (cerebral ganglion + suboesophageal ganglion + ventral nerve cord, VNC) and other tissues such as the muscle, silk gland and hemolymph.

The total free amino acid levels in the tissues were estimated by the method of Moore and Stein (1954), in 1% homogenate of the CNS, 2% homogenates of muscle and silk gland, and 0.05 ml of hemolymph in 10% trichloroacetic acid (TCA). The AAT and AIAT activity was assayed by the method of Reitman and Frankel (1957) in 2, 5 and 10% homogenates of the CNS, muscle and silk gland respectively, and 0.5 ml of hemolymph.

Soluble proteins were estimated by precipitation with equal volume of 10% TCA, which were then separated by centrifugation.

Total protein content was estimated by the method of Lowry *et al* (1951) from the precipitate obtained from tissue homogenates by 10% TCA.

3. Results

The total free amino acid levels declined from the early fourth instar to mid-fifth instar and were elevated significantly during the late fifth instar (table 1). The extent of change was found to vary among the 4 tissues examined. The decrease was 51,

Table 1. Changes in the total free amino acid content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	36.40	38.28	34.63	33.9	28.40	18.03	46.26
	SD	±0.96	±0.82	±0.96	±1.1	±1.42	±0.56	±0.89
	PC		+5*	-9**	-2*	-16***	-37***	+157***
Muscle	Mean	15.41	16.34	8.38	8.09	8.38	7.27	47.10
	SD	±0.81	±0.32	±0.46	±0.39	±0.21	±0.84	±0.59
	PC		+6.0*	-49***	-3*	+4*	-13*	+548***
Silk gland	Mean	33.47	35.55	45.2	32.4	31.77	18.55	52.57
	SD	±2.03	±0.45	±3.54	±4.7	±3.27	±0.28	±1.65
	PC		+6*	+27***	-28**	-2*	-42***	+183***
Hemolymph	Mean	21.22	24.25	12.45	11.73	9.73	6.47	37.66
	SD	±0.41	±1.1	±0.31	±0.86	±0.86	±0.37	±0.73
	PC		+14***	-49***	-6*	-17**	-34***	+482***

Each value, expressed as μmol of tyrosine/g wet wt. of tissue or 1 ml of hemolymph is a mean \pm SD of 4 separate samples, each containing the tissue from 15–20 animals. The per cent changes (PC) (+ or -) following the means and SD for each period were calculated taking the immediately preceding period as the control.

T test: *** $P < 0.001$; ** $P < 0.01$; *statistically not significant.

55, 45 and 70% in the CNS, muscle, silk gland and hemolymph, respectively from the early fourth instar to the mid fifth instar. The increase at the late fifth instar was 27, 206, 57 and 78% in the CNS, muscle, silk gland and hemolymph respectively. The decrease was maximum in the silk gland (42%) while the increase was higher in the muscle (548%).

The activity levels of AAT and ALAT showed considerable variations during the metamorphosis of *B. mori* (tables 2, 3). These variations were minor and not significant during the fourth instar, while they were significant during the fifth instar (tables 2, 3). In all the tissues studied this trend of change in these enzyme activities was more or less similar.

During the molting period a general decrease of enzyme activity was recorded in all the tissues studied (table 2). With the exception of a 11% decrease during the late

Table 2. Changes in the activity levels of AAT during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	0.99	0.96	0.85	0.67	1.10	0.87	0.55
	SD	±0.02	±0.01	±0.02	±0.02	±0.04	±0.3	±0.02
	PC		-3*	-11***	-21***	+64***	-21*	+37*
Muscle	Mean	1.23	1.15	1.09	0.94	1.39	1.18	1.03
	SD	±0.04	±0.11	±0.03	±0.04	±0.04	±0.09	±0.01
	PC		-7*	-5*	-14***	+48***	-15**	-13***
Silk gland	Mean	1.20	1.24	1.27	0.69	0.86	1.27	1.34
	SD	±0.23	±0.02	±0.09	±0.08	±0.03	±0.09	±0.26
	PC		+3*	+2*	-46***	+25**	+48***	+6*
Hemolymph	Mean	5.65	5.61	5.58	5.22	6.44	2.73	2.11
	SD	±0.09	±0.03	±0.04	±0.07	±0.10	±0.55	±0.14
	PC		+1*	-1*	-6**	+23***	-58***	-23***

Values are expressed as μmol of pyruvate formed/mg protein/h. The remaining notation is the same as in table 1.

Table 3. Changes in the activity levels of ALAT during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	1.30	1.27	1.26	1.04	2.41	1.07	0.86
	SD	±0.03	±0.02	±0.25	±0.03	±0.06	±0.05	±0.02
	PC		-2*	-1*	-17*	+132***	-56***	-20***
Muscle	Mean	1.62	1.44	1.21	1.08	2.05	1.51	1.30
	SD	±0.06	±0.07	±0.12	±0.06	±0.22	±0.03	±0.07
	PC		-11**	-16**	-11***	+90**	-26***	-14***
Silk gland	Mean	1.79	1.82	1.95	1.39	1.87	2.18	2.20
	SD	±0.18	±0.03	±0.33	±0.04	±0.12	±0.08	±0.19
	PC		+2*	+7*	-29**	+35***	+17**	+4*
Hemolymph	Mean	5.22	4.96	4.20	3.07	1.62	1.39	1.30
	SD	±0.07	±0.16	±0.68	±0.58	±0.16	±0.04	±0.28
	PC		-5*	-15*	-27*	-47***	-14*	-50***

Notations are same as in table 2.

fourth instar, the AAT activity more or less remained constant in the CNS during the fourth instar. The silk gland recorded maximum decrease (46%) followed by the CNS and muscle, while the hemolymph showed a negligible decrease (6%). All the tissues exhibited a significant increase in the AAT activity from the molting period to the early fifth instar. Following this, the tissues recorded a decrease during the middle and late fifth instar. The sole exception to this decrease was the silk gland which showed an increase during this period (table 2).

The changes in AIAT activity were largely similar to those in AAT activity. In CNS, muscle and hemolymph the activity decreased continuously from early fourth instar through the molting period. The activity then showed an increase in these tissues during early fifth instar, but decreased from there during middle and late fifth instar (table 3). Contrary to this pattern the AIAT activity in the silk gland increased from early to the late fourth instar and decreased during the molting period. From then the activity in this organ increased continuously during early to late fifth instar (table 3). The changes in activity in all the tissues from early to late fourth instar were only minor and statistically not significant.

Thus, in general, the AAT and AIAT activities exhibit: (i) little or no change during the fourth instar, (ii) a decrease during the molting period and (iii) an initial increase and a later decrease during the fifth instar. The silk gland provided an exception to this general trend.

Both total and soluble proteins registered an increase from the early fourth instar to the late fifth instar in all the tissues studied (tables 4, 5). However, the level of soluble proteins in the silk gland started declining from the molting period and continued to decrease thereafter. Maximum increase in the protein levels was recorded in the muscle and silk gland.

4. Discussion

It is known that silk-formation is largely controlled by the level of amino acid reserves supplied from the degenerating tissues (Noguchi *et al* 1974). Probably, silk-

Table 4. Changes in the-total protein content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	75.31	77.02	79.35	70.95	81.87	85.65	88.17
	SD	±4.2	±0.44	±7.3	±3.3	±8.8	±6.1	±2.3
	PC		+2*	+3*	-11*	+15*	+5*	+3*
Muscle	Mean	58.65	100.92	137.61	222.75	268.93	323.98	342.88
	SD	±1.92	±6.54	±9.17	±7.76	±11.4	±21.75	±23.7
	PC		+72***	+36***	+62***	+21***	+20**	+6*
Silk gland	Mean	92.68	162.52	203.86	247.48	308.35	371.33	374.13
	SD	±4.27	±22.07	±5.65	±7.51	±11.22	±10.26	±14.82
	PC		+75***	+25**	+21***	+25***	+20***	+1*
Hemolymph	Mean	10.87	11.09	11.57	18.28	28.59	30.47	33.21
	SD	±0.18	±0.27	±0.32	±0.23	±3.57	±1.23	±1.16
	PC		+2*	+4*	+58***	+56***	+7*	+9**

Values are expressed as mg protein/g wt. of tissue or 1 ml of hemolymph. Remaining notation is the same as in table 3.

Table 5. Changes in the soluble protein content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	27.52	32.98	35.45	37.52	42.16	49.33	50.15
	SD	± 1.97	± 1.05	± 0.7	± 1.97	± 1.07	± 1.82	± 2.0
	PC		+20***	+8**	+6*	+12**	+17***	+2*
Muscle	Mean	32.17	33.65	34.73	35.46	36.23	37.39	42.96
	SD	± 1.35	± 0.7	± 1.44	± 2.4	± 2.5	± 0.45	± 3.07
	PC		+5*	+3*	+2*	+2*	+3*	+15**
Silk gland	Mean	56.97	79.69	108.05	104.96	87.47	60.65	42.92
	SD	± 1.24	± 1.93	± 5.9	± 2.43	± 5.9	± 2.28	± 1.72
	PC		+40***	+36***	-3*	-17***	-31***	-29***
Hemolymph	Mean	10.87	11.06	11.57	18.28	28.59	30.47	30.75
	SD	± 0.18	± 0.8	± 0.32	± 0.23	± 3.57	± 1.23	± 1.16
	PC		+2*	+5*	+58***	+56***	+7*	+1*

Notations are same as in table 4.

production is also controlled by the hemolymph amino acids, besides their role in osmoregulation and homeostasis (Anderson 1984). The concentration of free amino acids is known to be influenced by various factors such as the changing dietary conditions (Anderson 1984), proteolytic action of the molting fluid on the old cuticle (Wyatt *et al* 1956), age, nutrition and climatic factors (Inokuchi 1970). Presumably, the fluctuating levels of amino acids in *B. mori* reflect general changes in the metabolism during metamorphosis. The increase in amino acid pool at the late fifth instar is reflective of the initiation of proteolytic activity, which is the characteristic feature of the pupal stage.

The aminotransferases (AAT and AIAT) mediate the transfer of amino groups of the amino acids to α -oxo-glutarate, oxaloacetate and pyruvate to form glutamate, aspartate and alanine respectively (Lehninger 1978). The elevation of AIAT and AAT activity levels of the tissues at the beginning of the instar and of the silk gland at the late fifth instar is indicative of increased amino acid turnover and glutamate formation. The decrease in their activity in the CNS, muscle and hemolymph at the late fifth instar is suggestive of their low utilization, thereby increasing the free amino acid concentration in these tissues.

The decrease in the activity levels of AAT and AIAT during the molting period denotes decreased mobilization of amino acid pool required for protein synthesis due to low metabolic activity. It is also likely that the decrease in biochemical constituents during the molting period is due to their loss in body fluids and exuviae (Gakhar and Maleyvar 1985). Simultaneous decrease of transaminase activity in all the tissues except the silk gland, indicates that the focus is on the silk gland during the later half of the fifth instar.

The present study indicates continuous protein synthesis during the metamorphosis of *B. mori*. Several workers have examined the variation in protein content during insect metamorphosis (Mansingh and Smallman 1967; Firling 1977). As supposed by Mansingh and Smallman (1967) the increase in the proteins of the CNS during metamorphosis may be correlated with the increase in cholinergic enzyme activity. Presumably, the increase in soluble proteins in the CNS is

attributable to the synthesis and storage of various enzymes and neurohumoral factors to be made available to the needy tissue during metamorphosis. Similarly, the increase in the level of total proteins probably accounts partially for building the CNS during development.

As reported in other insects (Martin *et al* 1971; Price 1973) the silkworm hemolymph may act as a transitory storage medium for proteins, which might migrate from other tissues such as the fatbody, muscle, CNS, etc. The muscle, being the contractile machinery of the animal, contains high protein levels in order to maintain its structural and functional integrity. The increase in the protein content of the silk gland is evidently due to high rate of fibroin synthesis (Sarangi 1985). The soluble proteins of the silk gland are probably mobilized into the formation of fibroin as evidenced by their decrease during metamorphosis. The fact that the salivary gland of *Calliphora* (Martin *et al* 1971) absorbs the proteins from the hemolymph, suggests a similar mechanism in *B. mori* between the silk gland and hemolymph, since the former is a modification of the salivary gland.

Thus the biochemical constituents studied in the present investigation seem to fluctuate, and undergo transformations that are necessary for an orderly sequence of events during silkworm metamorphosis. Presumably the CNS coordinates all such events during metamorphosis. The observation that certain neurohumoral factors such as the juvenile hormone (Kwangtung College of Agriculture and Forestry 1975) and β -ecdysone (Tai-chu-ying *et al* 1982) could elevate the transaminase activity, provides evidence as to the central nervous control of metabolism. It is probable that the transaminase activity is enhanced by such factors, which in turn increases the amino acid turn-over in the silk gland for fibroin synthesis. It is not known if such a situation exists in *B. mori*, but it presumably does.

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Interrelationship between the states of chromosome compaction, replication and transcription patterns in the polytene-X chromosome of *Drosophila melanogaster*

D MUTSUDDI*, MAUSUMI MUTSUDDI (NEE DAS)
and A K DUTTAGUPTA

Genetics Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

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Abstract. We studied by autoradiography the replication and transcription patterns of polytene X chromosomes in different segmental aneuploid and hyperploid conditions in a constant diploid autosomal background. In all karyotypes, the patterns of X chromosome compaction and DNA synthesis were observed to be set strictly either at a 'male-level' or a 'female-level'. Addition of X-fragments to 1X progeny exceeding a certain limit (62% with proximal duplication) changed the pattern of chromatin compaction and replication of X chromosomes from male to female level, while similar additional X-fragments had no comparable effect on 2X hyperdiploids. In contrast, different levels of X-transcription per segment were observed in these aneuploids with variable X-load and in consequence, an equivalent amount of total X-transcription (relative to autosomal transcription) was visualized. These findings while favour a positive correlation between the state of chromosome compaction and replication patterns, are at odds with the idea that for an altered level of transcription, a modulation in the state of chromosome compaction and replication is an obligatory pre-requisite.

Keywords. *Drosophila melanogaster*; X-segmental aneuploids; hyperploids; chromosome compaction; replication; transcription.

1. Introduction

Dosage compensation for differences in the number of X-linked gene copies in *Drosophila* is achieved by transcriptional modulation, enhancing the level of activity in males (Mukherjee and Beermann 1965; Gadagkar *et al* 1982). Decondensed nature and early replicating properties of the X chromosomes were observed to be concomitant with its hyperactive nature (Lakhotia and Mukherjee 1970; Das *et al* 1982; Mutsuddi *et al* 1985), and thus was speculated to have some unspecified role in elevation of the level of X-transcription in heterogametic sexes. Yet a causal connection between them has not been proved.

Diploid complements with more than one but less than two X chromosomes were constructed to study the regulation of X-encoded gene function in *Drosophila* (Stewart and Merriam 1975; Maroni and Lucchesi 1980; Chatterjee 1985). Unfortunately, conclusions reached through these studies were divergent and equivocal. While 'autosomally encoded, rate limiting, positive regulator' model (Lucchesi 1977) was found to be best suited to explain the findings of Maroni and Lucchesi (1980), the idea that 'dosage compensation is primarily a property of X-organization itself' was endorsed from Chatterjee's (1985) observations. The latter idea is, however, contradicted by Devlin *et al* (1984) and Lucchesi and Manning

*To whom correspondence should be addressed.

(1987). In this background, we looked into the functional properties of the polytene-X chromosomes in different segmental aneuploid and hyperploid conditions in a constant diploid autosomal background to test the relationship between chromosome compaction, replication and transcription patterns.

2. Materials and methods

2.1 Genetic crosses

X-chromosomal segmental aneuploids and hyperploids were constructed using different X;Y translocation stocks, viz. B29, J8 and B44, where the X chromosomes were broken at 4C, 8C and 11A respectively (Steinmann-Zwicky and Nöthiger 1985). The males of these stocks contained a free Y and a translocated Y chromosome, the long arm (Y^L) of which was attached to the distal, and the short arm (Y^S) to the proximal segment of X chromosome. The X chromosome was marked with yellow (y), but covered with a corresponding y^+ duplication attached to the long arm (Y^L) of the translocated Y. The short arm (Y^S) of translocated Y was marked with B^S (figure 1a,b). The terms 'distal' and 'proximal' designate the position of chromosomal segments relative to the chromocenter, that is located to the right of region 20F (of Bridges' map). For a detailed description of genetic markers see Lindsley and Grell (1968).

Different segmental aneuploid and hyperploid larvae were constructed by crossing males of the aforesaid translocation stocks to yellow (y) and attached-X [$C(1)RM, y\ pn/Y$] virgin females, respectively (figure 1a,b; also see Stewart and Merriam 1975; Maroni and Lucchesi 1980). Mature third instar larvae of desired classes were selected for experimental purpose. The karyotypes of expected classes were also confirmed by examination of polytene chromosomes. Oregon R^+ males and females served as control.

2.2 Autoradiography

For replication study salivary glands of desired larvae were incubated in 500 $\mu\text{Ci/ml}$ of [^3H] thymidine ([^3H] TdR, specific activity: 17.4 Ci/mM, Bhabha Atomic Research Centre, Trombay, Bombay) for 20 min. Squashed preparations were covered with Kodak AR 10, exposed for 3 weeks, developed in Kodak D19b and stained in toluidine blue. For transcription studies, dissected glands were incubated in 400 $\mu\text{Ci/ml}$ of [^3H] uridine ([^3H] UR, specific activity: 12.7 Ci/mM, Bhabha Atomic Research Centre, Trombay, Bombay) for 10 min. Exposure time was two weeks (also see Das *et al* 1982).

2.3 Sequencing of [^3H]TdR labelling patterns

[^3H]TdR pulse labelled larval salivary gland chromosomes of *Drosophila* revealed different patterns of silver grain distribution over different chromosomal subunits (i.e. puffs, interbands and bands) (Lakhotia and Mukherjee 1970; Das *et al* 1982). Taking chromocentric labelling pattern (which is always very late replicating) as an index, different patterns were classified as (i) initial or dispersed discontinuous or

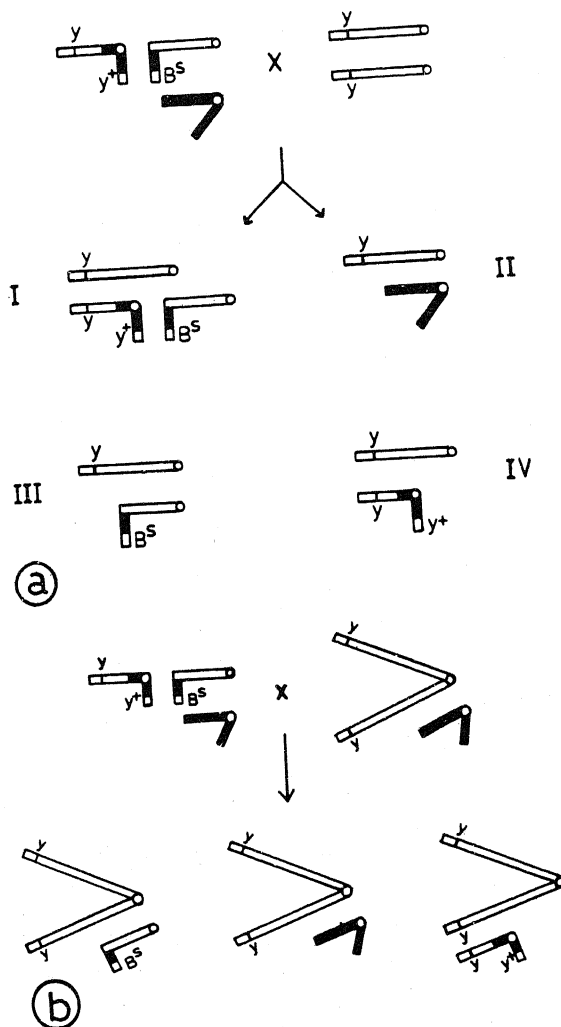


Figure 1. Scheme of genetic crosses used to generate X chromosomal segmental aneuploid (a) and hyperploid (b) larvae. Hollow and solid bars represent X and Y chromosomes respectively; open circles are centromeres. The genetic markers cited here are explained in the text. Different T(X;Y) males were crossed to (a) yellow (y), and (b) attached-X [C(1)RM, y pn/Y] virgin females to yield different segmental aneuploid and hyperploid larvae respectively. Selection of larvae was made following the methods of Stewart and Merriam (1975) and Maroni and Lucchesi (1980).

DD (labelling mostly on puffs and interbands), (ii) middle or continuous or C (labelling throughout the chromosomes), and (iii) terminal or discontinuous or D (labelling mostly on bands) stages. On the basis of number of sites labelled and the intensity of labelling on them, each stage was further subdivided and arranged in the order of DD1-DD2-DD3-1C-2C-3C-3D-2D-1D stages (also see Das *et al* 1982).

2.4 Frequency of labelling

For detailed sitewise analysis, 45 and 20 replicating units on 1A to 12DE

subdivisions of the X chromosome and 56F to 60F subdivisions of the 2R of Bridges' map (Lindsley and Grell 1968), respectively were considered (Lakhotia and Mukherjee 1970). Silver grains over all labelling sites were counted from late continuous to very late discontinuous stages. To consider any site as labelled, a minimum of 4 grains was taken as the lower limit. The frequency of labelling of a site was determined by the number of times a site was labelled among all labelled nuclei examined.

3. Results

3.1 *Functional morphology*

In polytenized cells, 1X aneuploids were observed to exhibit pale stainability and puffy nature in both their entire and duplicated X chromosomes (relative to autosomes) as long as the duplication size remains within the 62% limit from the proximal end (figure 2a). In these aneuploids, the distal non-duplicated and proximal duplicated segments represented a diameter, equal to and twice the cross-sectional area of paired autosomal regions respectively.

Interestingly, addition of further X-fragments exceeding this 62% limit (so far studied), dramatically shifted all the properties of X chromosomes from 'male-like' to 'female-like'. In 1X larvae with 82% proximal X-duplication (dp. 4D-20F), both homologues of X chromosomes (entire and duplicated ones) had a 'female-like' appearance, i.e. had a diameter and staining intensity identical to those of asynapsed autosomal arm of the same complement (figure 2b).

In 2X individuals, addition of X fragments, regardless of their size and location, only increased the total diameter of duplicated hyperploid regions to 1.5 times the cross-sectional area of its non-duplicated regions, which had a diameter comparable to that of paired autosomes. In these hyperploids, all portions of X chromosomes represented a staining intensity reasonably similar to that of their normal diploid counterpart in homogametic sexes (figure 2c,d).

3.2 *DNA synthesis in the X element*

In all aneuploids and hyperploids, the X chromosomes were observed to display a pattern of DNA synthesis expected either for a male-X or a female-X chromosome. For example, in 1X individuals having X-fragments up to 62% as proximal duplication (dp. 8D-20F), both the entire and duplicated X chromosomes followed a typical 'male-like' asynchronous pattern of replication as compared to autosomes (figure 3a). In contrast, in aneuploids having a fragment size exceeding this 62% limit proximally (figure 3b) and in all hyperploids (figure 3c-f), the X chromosomes displayed a synchronous pattern of replication with the autosomes, typical for X chromosomes in female cells. Table 1 records that the labelling frequency of each X chromosomal sites in aneuploids with up to 62% proximal duplications (dp.8D-20F) exhibited a close correspondence with that of respective unit in euploid males, while similar X-units in other aneuploids (dp.4D-20F) and all hyperploids showed a close similarity with that of corresponding units in euploid females.

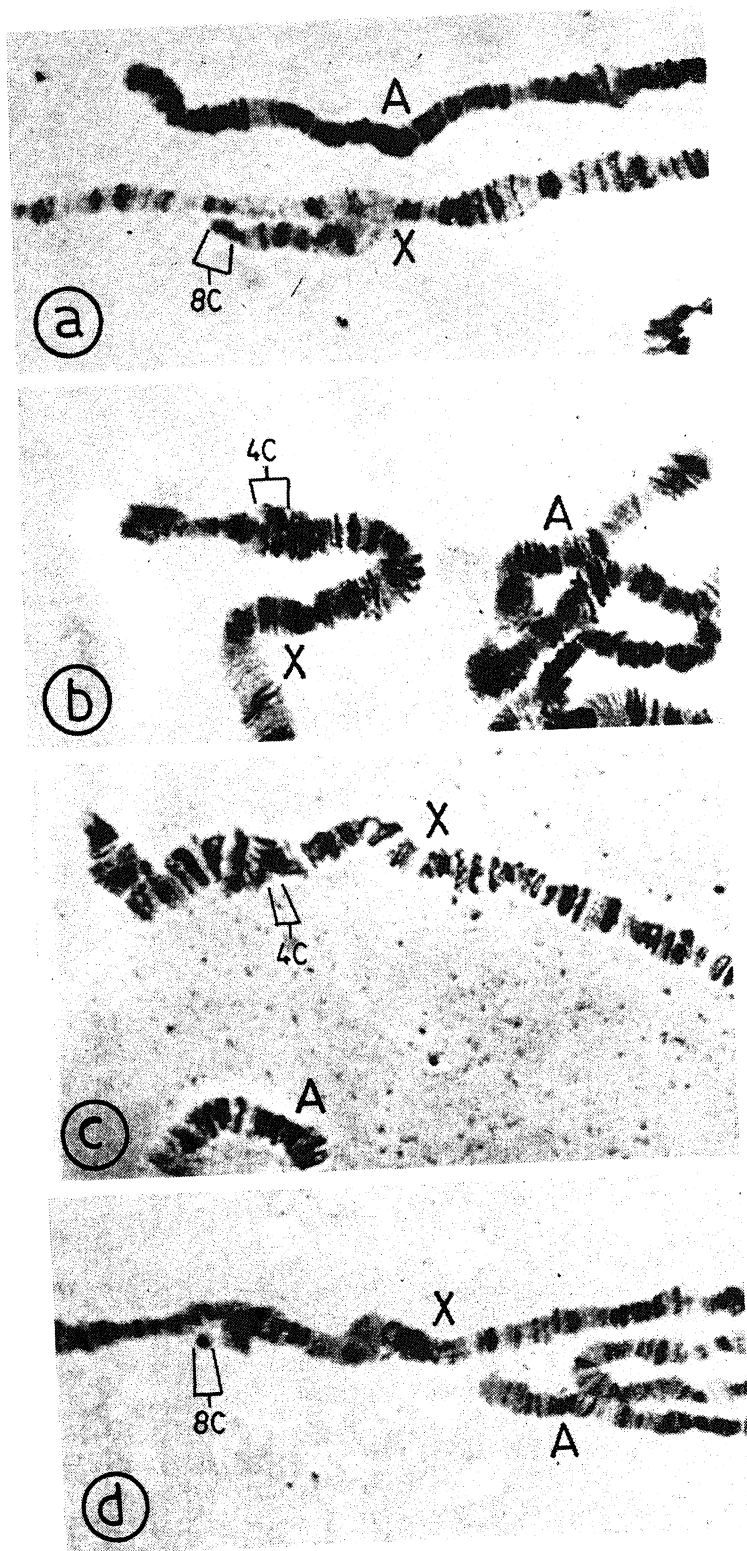


Figure 2. For caption, see p. 386.

Table 1. [³H]TdR labelling frequency of 22 replicating sites on X chromosome in different segmental aneuploids and hyperloids as compared to that of wild type males (1X;2A) and females (2X;2A). In aneuploids and hyperloids, size of duplicated fragments was estimated on the basis of extent of cytological map (Bridges') included in the segment. The number of nuclei examined for each genotype was 33.

Individuals with different X chromosomal load										
Repli- cating unit	Chromo- somal site	1X	1-50X (dp-11A-20F)	1-62X (dp-8D-20F)	1-82X (dp-4D-20F)	2X	2-18X (dp-1A-4C)	2-38X (dp-1A-8C)	2-62X (dp-8D-20F)	2-82X (dp-4D-20F)
1	1A	73	76	76	93	98	100	96	100	100
2	1DEF	26	26	26	48	42	45	42	42	45
3	3A	9	9	9	20	20	24	20	20	24
4	3C	98	100	100	93	92	96	96	96	96
5	3DE	28	26	24	45	50	48	58	48	45
6	4A	26	26	23	50	45	42	48	45	50
7	4BC	45	42	42	87	72	75	71	71	71
8	4DEF	68	66	63	100	100	100	100	100	100
9	5A	18	21	21	45	45	42	39	45	48
10	5CD	24	24	24	54	57	60	60	51	54
11	6A	42	39	39	97	97	95	95	97	95
12	7ABC	80	80	76	93	96	93	100	100	100
13	7E	28	27	27	57	60	63	63	54	54
14	8ABC	48	50	70	85	90	87	87	93	93
15	8E	33	30	39	60	66	60	63	69	69
16	9A	40	40	42	100	97	93	96	96	100
17	10A	30	30	33	63	70	69	69	73	76
18	10B	20	21	24	84	88	90	84	90	87
19	11A	100	100	100	100	100	100	100	100	100
20	11CD	9	9	6	45	45	39	39	45	48
21	12A	33	30	39	74	75	74	78	81	78
22	12DE	98	100	100	100	98	100	100	100	100

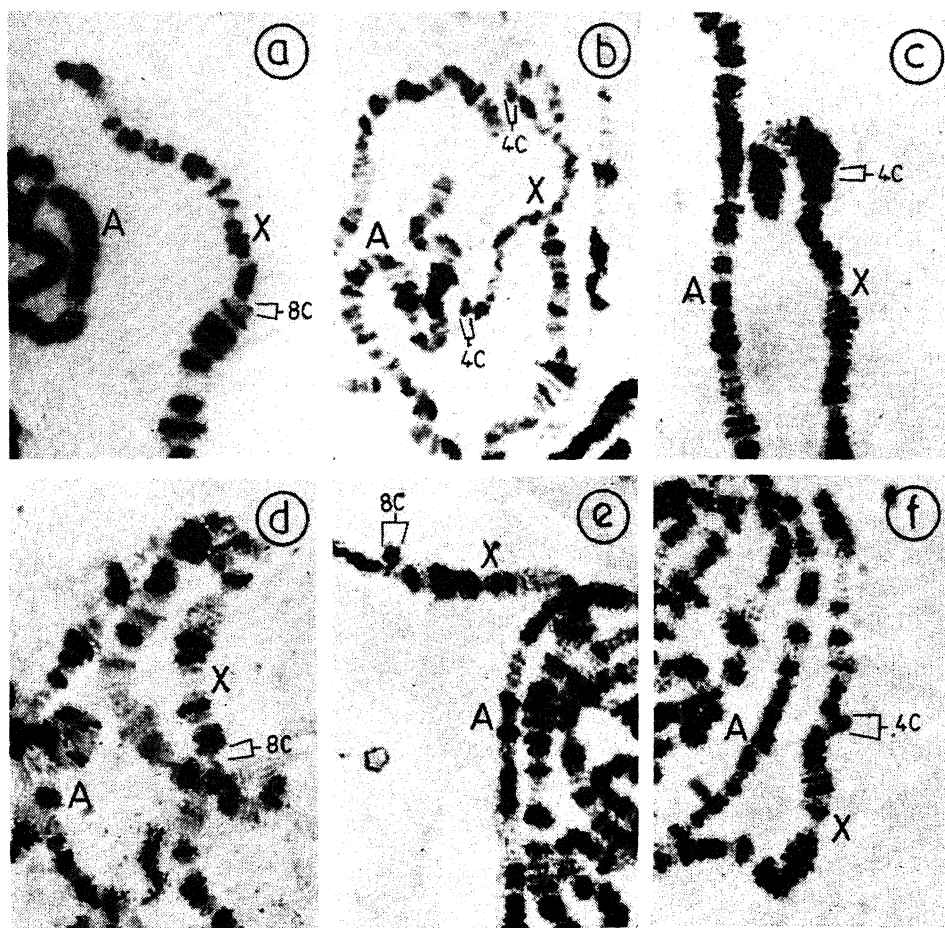


Figure 3. Autoradiograms showing $[^3\text{H}]\text{TdR}$ labelling patterns on polytene-X chromosomes (X) as compared to those on autosomes (A) in different segmental aneuploid (a-b) and hyperploid (c-f) situations, duplicated for the segment 8D-20F (a), 4D-20F (b), 1A-4C (c), 1A-8C (d), 8D-20F (e) and 4D-20F (f). Blocks denote end of duplicated X-fragments.

3.3 RNA synthesis in the X element

The mean grain number over the whole X chromosome (1A-20F) as well as different X-segments, relative to that of a part of an autosome (56F-60F of 2R), in these aneuploids and hyperploids was examined and compared with that of normal euploid sibs. Data revealed that the mean grain ratio over the whole X chromosome and autosome in aneuploids with 1.62 and 1.82 X-load was statistically insignificant from that of normal males ($P > 0.05$). Among hyperploids, similar comparison with euploid females also found the difference to be insignificant ($P > 0.05$, table 2). Segmental analysis of incorporation pattern (table 2) revealed that while the labelling density was increased in duplicated segments, it was decreased in non-duplicated one. The distribution of silver grains, in terms of

Table 2. Mean X/A ratios over different X-segments in different aneuploids and hyperploids as compared to those of euploid males (1X;2A) and females (2X;2A) respectively.

Karyotype	X-segment			Total	N
	1A-4C	4D-8C	8D-20F		
IX	0.72 ± 0.08 <i>t</i> = 1.054	1.20 ± 0.07 <i>t</i> = 0.058	2.31 ± 0.20 <i>t</i> = 0.928	4.231 ± 0.23 <i>t</i> = 0.763	15
1.62X (dp-8D-20F)	0.67 ± 0.22 <i>t</i> = 0.214	0.835 ± 0.17 <i>t</i> = 1.985	2.947 ± 0.21 <i>t</i> = 2.197*	4.45 ± 0.28 <i>t</i> = 0.604	15
1.82X (dp-4D-20F)	0.49 ± 0.04 <i>t</i> = 2.57*	1.135 ± 0.06 <i>t</i> = 0.705	2.41 ± 0.09 <i>t</i> = 0.456	4.035 ± 0.37 <i>t</i> = 0.45	14
2X	0.855 ± 0.10	1.205 ± 0.05	2.51 ± 0.08	4.57 ± 0.38	15
2.18X (dp-1A-4C)	0.98 ± 0.23 <i>t</i> = 0.498	1.17 ± 0.21 <i>t</i> = 0.162	2.465 ± 0.32 <i>t</i> = 0.136	4.615 ± 0.4 <i>t</i> = 0.082	13
2.38X (dp-1A-8C)	0.95 ± 0.11 <i>t</i> = 0.202	1.33 ± 0.08 <i>t</i> = 1.696	2.44 ± 0.24 <i>t</i> = 0.277	4.72 ± 0.45 <i>t</i> = 0.255	12
2.62X (dp-8D-20F)	0.845 ± 0.09 <i>t</i> = 0.074	1.07 ± 0.12 <i>t</i> = 1.038	3.03 ± 0.18 <i>t</i> = 2.64*	4.945 ± 0.33 <i>t</i> = 0.745	12
2.82X (dp-4D-20F)	0.825 ± 0.03 <i>t</i> = 0.287	1.32 ± 0.08 <i>t</i> = 1.219	2.94 ± 0.07 <i>t</i> = 4.045*	5.085 ± 0.24 <i>t</i> = 1.146	12

**P* < 0.05. N, Number of nuclei examined.

percentage (figure 4), over different X segments in these karyotypes also confirmed the above observation. However, increase in labelling density over duplicated regions was not always proportional to the magnitude of duplication.

4. Discussion

In the present investigation, the pattern of X chromosome compaction in all karyotypes was observed to be set strictly either at a 'male-level' or at a 'female-level'. Addition of X-fragments to 1X individuals exceeding a certain limit changed that state of chromosome compaction from male to female level, while similar duplications on 2X individuals had no comparable effect. The microphotographs of X chromosomes in different aneuploids published earlier (Maroni and Lucchesi 1980; Prasad *et al* 1981; Chatterjee 1985; Prasad-Sinha and Mukherjee 1985; Mukherjee and Ghosh 1986), also show similar effects. These and other (Belote and Lucchesi 1980; Lucchesi and Skripski 1981) findings demand that the pattern of chromatin packing set up by the X chromosomes in euploid females be viewed as 'basal level', and any further condensation is 'unsuitable for correct functioning of transcription apparatus' (Devlin *et al* 1984).

The relative duration of DNA synthesis of X chromosomes in these abnormal karyotypes was found to be positively correlated with its degree of chromosome compaction. The rule that may be applicable to account such relationship is that: more decondensed state of chromosome, earlier the termination of replication. Earlier findings (Mutsuddi *et al* 1984, 1985, 1988a) also favour such a relationship.

Regarding transcription, present results are compatible with the idea that different quantum of X chromosome in a constant autosomal background does not interfere the total transcription for this chromosome (Maroni and Lucchesi 1980).

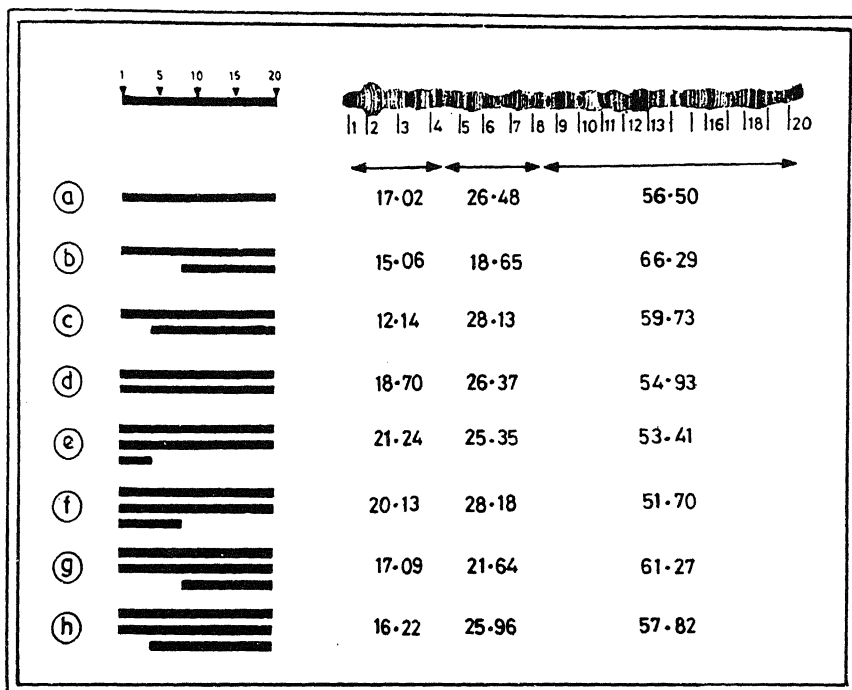


Figure 4. Diagrammatic representation of [^3H]UR silver grain distribution pattern, in terms of percentage, over different X-segments (viz. 1A-4C, 4D-8C and 8D-20F) in individuals having the X chromosomal load (a) IX, (b) 1-62X (1X + dp-8D-20F), (c) 1-82X (1X + dp-4D-20F), (d) 2X, (e) 2-18X (2X + dp-1A-4C), (f) 2-38X (dp-2X + dp-1A-8C), (g) 2-62X (2X + dp-8D-20F) and (h) 2-82X (2X + dp-4D-20F).

Thus, the finding of different levels of X-transcription against only two alternate states of chromosome compaction and replication fits better with the view that the structural organization (state of chromatin compaction) of a chromosome does not always reflect the functional behaviour of the same (Devlin *et al* 1984), rather than with the thought of its obligatory role (Chatterjee 1985). Experimental observations of Eissenberg and Lucchesi (1982, 1983) and Mutsuddi *et al* (1988b) provide instances in favour of our argument.

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Figure 2. Photomicrographs showing morphology of polytene-X chromosomes in different segmental aneuploid (a–b) and hyperploid (c–d) conditions, duplicated for the segment 8D–20F (a), 4D–20F (b), 1A–4C (c) and 8D–20F (d). Blocks denote end of duplicated X-fragments. X, X chromosome; A, autosome.

Effect of formulated feeds on the amylase activity and growth in three species of carps

S V PHADATE and L N SRIKAR

Department of Biochemistry, College of Fisheries, Mangalore 575 002, India

MS received 3 July 1989; revised 20 July 1990

Abstract. Assay of amylase activity carried out in 3 species of carps viz. *Cyprinus carpio*, *Catla catla* and *Hypothalmichthys molitrix*, when fed with 3 formulated feeds viz. protein, lipid and carbohydrate rich diets, showed an increase in the enzyme activity discernible after 15 days of feeding. The activity decreased during the starvation period. Protein rich diet ensured better growth in fishes than the lipid and carbohydrate rich diets.

Keywords. *Cyprinus carpio*; *Hypothalmichthys molitrix*; *Catla catla*; amylase; feeding; starvation.

1. Introduction

The presence of amylase in different organs viz. pancreas, pyloric caeca, intestine, liver and bile have been demonstrated by many workers (Sarbah 1951; Chiu and Benitez 1981; Hofer 1982; Bitterlich 1985; Sturmbauer and Hofer 1986). Though data on pH and temperature optima for enzyme activity are available, information on the effect of feeding formulated diets on the activity of amylase is scanty. The effects of different carbohydrates on the activity of amylase in *Brylton melanopteres*, carp and trout have been worked out by Reimer (1982) and Hofer and Sturmbauer (1985). The present paper describes the effect of 3 formulated feeds on the amylase activity and growth in the common carp *Cyprinus carpio*, catla *Catla catla* and silver carp *Hypothalmichthys molitrix*.

2. Materials and methods

Fingerlings (55 each) of *C. carpio*, *C. catla* and *H. molitrix* obtained from the State Govt. Fish Farm, Gaznoor, were stocked separately in aerated glass aquaria (300 l) in duplicate sets and fed *ad libitum* twice daily at 9000 and 1700 h for 60 days with 3 different artificial pelleted feeds viz. protein, carbohydrate and lipid rich feeds prepared according to the composition given by Reimer (1982). Samples were drawn before the commencement of the feeding (control), and at 5 day intervals during the feeding period of 60 days and post feeding starvation respectively. Whole gut was used for assay. The enzyme was extracted using glass distilled water (20 ml/g gut). Amylase activity was determined by the method of Sumner (1924) using 1% starch as substrate. The activity was calculated on the basis of maltose released in 5 min by hydrolysis of starch. One unit of amylase activity (AU) was defined as the mg of maltose liberated by 0.1 ml extract at 20°C in 5 min.

3. Results and discussion

Amylase activity increased throughout the feeding period in *C. carpio* fed on

protein rich diet except on 10th and 50th days (figure 1). During the first half of starvation period the decrease in the activity was moderate, while it was very steep during the second half. In the case of fish fed with carbohydrate rich diet, initially the activity fluctuated during the first 10 days and increased during the subsequent feeding period. A maximum value of 0.437 AU was recorded on the 40th day. The activity decreased by 0.138 AU during the 10 days of starvation. Almost a similar trend was observed in the enzyme activity in fish fed on lipid-rich feed. However, till the first 20 days, wider fluctuations were observed. On starvation the activity decreased only marginally during the first 5 days, while during the next 5 days there was a sharp decline.

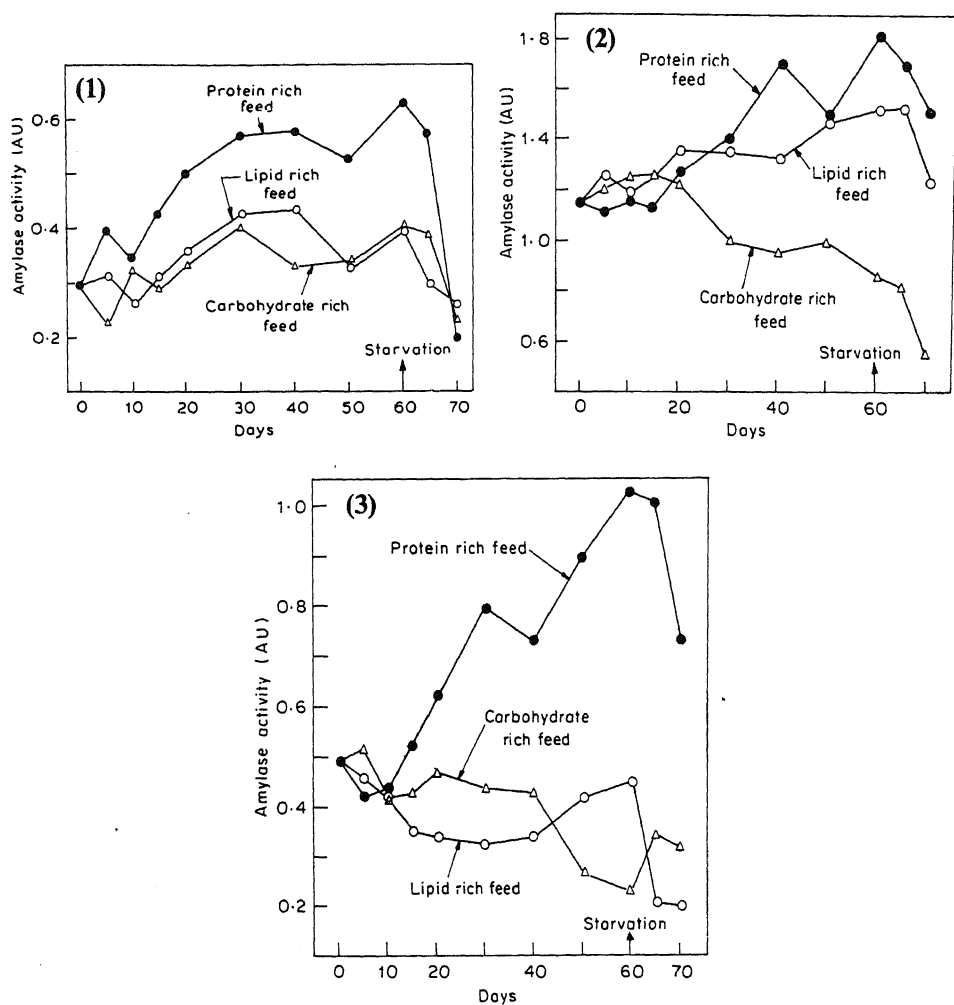
In *C. catla* fingerlings fed on protein-rich diet, amylase activity showed very little variation during the first 20 days. The activity increased thereafter till 40th day, decreased on 50th day and increased again on 60th day (1.843 AU). The activity decreased slightly during the starvation period (figure 2). After the initial increase till 15th day, the activity in fish fed on carbohydrate rich feed was found to decrease till the end of feeding period reaching a value of 0.876 AU. During starvation period also, the activity decreased. In fish fed on lipid rich diet an overall increase during the feeding period (0.381 AU) was noticed. During starvation, the activity increased slightly during the first 5 days, while it decreased rapidly during next 5 days.

Figure 3 represents the effect of feeding different diets on amylase activity in *H. molitrix*. A total increase of 0.526 AU could be observed at the end of 60 days feeding period in fish fed on protein-rich feed. During the starvation period, the activity decreased considerably. The enzyme activity in fish fed on carbohydrate-rich feed showed a decrease right from the start of the experiment till 30th day of feeding, and increased thereafter. During starvation, the activity decreased by 0.249 AU within 10 days. A decrease in enzyme activity was noticed in fish fed on lipid rich feed also. However, the activity increased sharply during the first 5 days of cessation of feeding and then slightly during subsequent 5 days of starvation.

The highest growth was observed in fish fed with protein rich diet followed by lipid rich diet and carbohydrate rich diet in all the 3 species (figure 4). Among the 3 species, fingerlings of *H. molitrix* showed the maximum growth among the 3 feeds tested. The increment in weight over the initial for the protein rich diet were 98.1% in *C. carpio*, 133.9% in *C. catla* and 134% in *H. molitrix*. The corresponding values for lipid rich feed were 69.6, 110.8 and 112.6% and for carbohydrate rich diet 53.8, 45.9 and 98.1% respectively. During starvation a loss in weight was observed in all the 3 species.

In the present study, definite trend in enzyme activities were discernible only after 15 days which indicate the time requirement for metabolic adjustments to the feeds. Kawai and Ikeda (1972, 1973) have observed definite trends in enzyme activities in *C. carpio* after 7 days and in trouts after 10 days of commencement of feeding.

Earlier workers have demonstrated that amylase activity is related to the carbohydrate quantity in the diets (Fal'ge *et al* 1978; Spannhof and Plantikow 1983; Hofer and Sturmbauer 1985). Inclusion of crude starch in the diet is known to reduce amylase activity as a result of adsorption of amylase to crude starch. In the present formulated feeds, fish meal was the main source of protein and all the fishes showed maximum growth on protein rich feed. The growth of common carp and silver carp on lipid rich and carbohydrate rich feeds was similar while in catla it was decidedly better in fish fed on lipid rich diet than those on carbohydrate rich



Figures 1-3. Effect of feeding and starvation on amylase activity in fingerlings of (1) *C. carpio*, (2) *C. catla* and (3) *H. molitrix*.

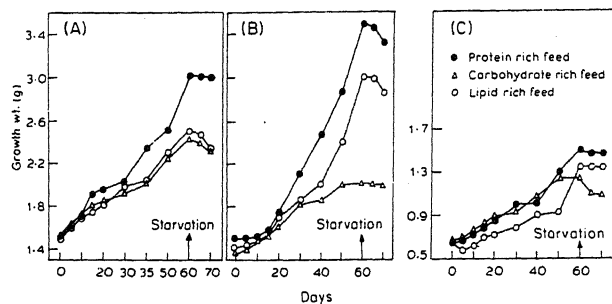


Figure 4. Effect of feeding and starvation on growth of fingerlings of (A), *C. carpio*, (B), *C. catla* and (C) *H. molitrix*.

diet. Compared to the lipid and protein rich feeds, growth of fish on carbohydrate rich diet was poor. Low growth rates on high starch diet have also been noticed in carps by Kawai and Ikeda (1973). Chiou and Ogino (1975) have attributed this to the type of starch. They found that the digestibility of β -starch was lower than that of α -starch and that digestibility of α -starch averaged 85%, regardless of starch levels, while that of β -starch decreased gradually as the starch level increased.

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*Not seen in original.

Biology of *Tetrastichus pantnagarensis* (Hymenoptera: Eulophidae) a hyperparasite of *Diaphania indica* (Lepidoptera: Pyralidae) through *Apanteles taragamae* (Hymenoptera: Braconidae)

CLEMENT PETER and B V DAVID

Fredrick Institute of Plant Protection and Toxicology, Padappai 601 301, India

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Abstract. *Tetrastichus pantnagarensis* Khan (Hymenoptera: Eulophidae) was recorded as a hyperparasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) through *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). Studies were conducted on its biology. This solitary endoparasite oviposited in the host pupa within the host cocoon and the larva passes through 3 instars. Mean development time from egg to adult is 12-18 days at $26.43 \pm 2.53^\circ\text{C}$. After a preoviposition period of 1-2 days oviposition continued for 7-10 days. Mean adult longevity for males was 8-51 days and for females 11-41 days.

Keywords. *Tetrastichus pantnagarensis*; hyperparasite; biology.

1. Introduction

The pumpkin caterpillar, *Diaphania indica* (Saunders) is a major pest of several cultivated cucurbits. During the course of the studies on the bioecology of *D. indica*, the braconid, *Apanteles taragamae* was recorded as its major gregarious endoparasite. It was recorded for the first time from Sri Lanka as a parasite of *D. indica* on snake gourd (Wilkinson 1931). In 1948, Bhatnagar listed 13 hosts of *A. taragamae* from India. At Padappai, *D. indica* is a major pest on *Coccinia grandis* (L.) Voight and during certain months 80% parasitism by *A. taragamae* was recorded. However the pest was not under effective biological control. It was observed that the cocoons of *A. taragamae* were attacked by a hyperparasite which was subsequently identified as *Tetrastichus pantnagarensis* Khan. Hyperparasitism is defined as any form of parasitism other than primary (Smith 1916). There is disagreement over the importance of hyperparasitism in biological control (Narayanan 1957; Flanders 1963; Valentine 1974) but it is generally conceded that the identification of hyperparasitism is supremely important to biological control. Hyperparasitism is more common in the Tetrastichinae and is sometimes obligatory. Genera of tetrastichines that include hyperparasitic species are *Tetrastichus* Walker, *Crataepiella*, *Domenichini* and *Melittoba* Westwood (Gordh 1988). *T. pantnagarensis* was originally described as a parasite of *Apanteles* sp. on the larvae of Bihar hairy caterpillar, *Diacrisia obliqua* Walker (Khan 1983). This is the first record of *T. pantnagarensis* as a hyperparasite of *D. indica* through *A. taragamae* and there is no information on biology of *T. pantnagarensis*. Therefore in the present investigation observations on the biology of this hyperparasite were carried out.

2. Materials and methods

To study the biology of the secondary parasite it was essential to maintain a culture of the primary parasite. The following procedure was adopted to rear *A. taragamae* in the laboratory.

Twenty-five third instar larvae of *D. indica* were released on a bouquet of *Coccinia* leaves inserted into a glass vial (6×1.5 cm) placed inside a plastic jar (12×10 cm) with wire mesh fitted lid. Three mated females of *A. taragamae* were released into each jar. The larvae were exposed to the parasite for 12 h. At the end of this period the parasites were removed from the jar and the larvae allowed to feed on the leaves. The leaves were changed periodically until the parasite larvae completed their development inside the host larvae and the parasite cocoons were formed. These cocoons were collected from the rearing jars and placed in specimen tubes.

Freshly formed *A. taragamae* cocoons were exposed to *T. pantnagarensis* adults in glass specimen tubes for 1 h. After this the adults were separated from the cocoons and 12 h later the cocoons were dissected and the host pupae taken out and dissected again. This was repeated at periodic intervals of 6 h, until hatching of the hyperparasite eggs was observed. After the incubation period, the cocoons were dissected every 24 h and sketched. To establish the larval period the parasitised host cocoons were dissected at periodic intervals using a Carl Zeiss Zoom Citoval-2-Stereomicroscope. To determine the shape and size of the larval mandibles the larvae were boiled in a 10 KOH solution for 45 s for clearing the host tissues. After washing in distilled water they were mounted in Hoyer's medium on microscope slides. The parasite larvae were measured with a calibrated ocular micrometer and sketched using a camera lucida attached to a Carl Zeiss Laboval 4 compound microscope. The prepupal and pupal periods were also recorded. The rearing was done at $26.43 \pm 2.53^\circ\text{C}$ and 65% RH.

The preoviposition and oviposition periods were determined by exposing the freshly formed parasite cocoons to the hyperparasite at intervals of 24 h beginning with the day of emergence. The cocoons were dissected after each exposure and the number of parasitised hosts as well as the number of eggs laid per host were counted. The host cocoons were exposed continuously until the female died.

Adult longevity was determined by feeding honey solution to freshly emerged males and females.

The stock culture of *T. pantnagarensis* was maintained in the insectary by rearing it on *A. taragamae* cocoons. The cocoons were exposed for 6 h after which the adults were separated from the host. The exposed cocoons were placed in glass specimen tubes until the adults emerged.

3. Results and discussion

3.1 Immature stages

3.1a Egg: The freshly laid egg is white, slightly curved in the middle and sausage-shaped (figure 1A–D). Eggs are found mainly in the abdomen of the host. The length of 20 eggs averaged 0.31 mm and the width in the middle averaged 0.07 mm

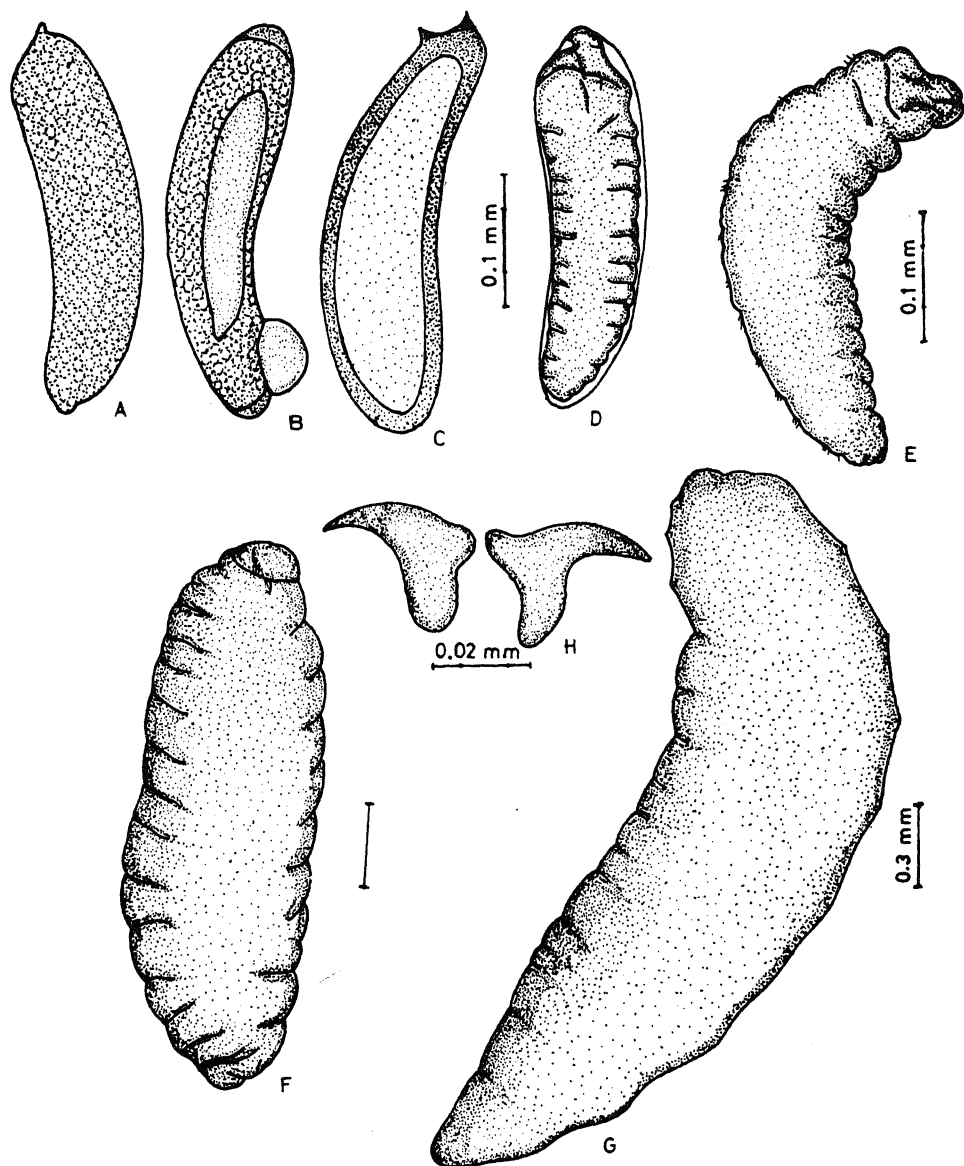


Figure 1. Developmental stages of *T. panthagarensis*, A-D, Egg in various stages of development; E, 1st instar; F, 2nd instar; G, 3rd instar; H, Mandibles.

(table 1). Initially the chorion is smooth and shining white but with development becomes transparent and the embryo can be clearly seen.

3.1b Larva: The larva passes through 3 instars. The first instar larva is white and fusiform. The body is broad at the anterior end and tapers at the posterior end. It is broadest in the thoracic region. The body consists of 13 segments. Two rows of minute setae are visible on the dorsal side of the body (figure 1E). First instar larva averaged 1.15 mm in body length and 0.37 mm in width. The mandibles at this

Table 1. Mean size and duration of immature *T. pantnagarensis* reared at $26.43 \pm 2.53^\circ\text{C}$.

Stage	n	Length (mm)	Width (mm)	Duration (day)
		$\bar{X} \pm \text{SEX}$	$\bar{X} \pm \text{SEM}$	
Egg	20	0.31 ± 0.05	0.07 ± 0.02	40–56 h
1st instar	20	1.15 ± 0.15	0.37 ± 0.10	1–2
2nd instar	18	1.69 ± 0.12	0.61 ± 0.18	1
3rd instar	15	2.57 ± 0.31	0.77 ± 0.09	1–2
Prepupa	12	1.65 ± 0.28	0.70 ± 0.61	1
Pupa	12	2.38 ± 0.26	0.76 ± 0.14	5–6

stage are unsclerotized, simple and curved. Each measures 0.014 mm in length and 0.007 mm at the base. Spiracles are absent. Tracheal trunks are visible from the second to the tenth segment but not well defined.

The second instar larva is smooth and flabby and dorsal setae are absent. Other external structures are not visible. The head is smooth and unsclerotized (figure 1F). The larva averages 1.69 mm in length and 0.61 mm in width at the middle. This instar is less active compared to the first instar. Tracheal system similar to first instar and spiracles are absent.

The third instar larva is robust, opaque and fusiform. The head is narrow and rounded and not chitinized (figure 1G). It is smooth, dirty brown and 2.57 mm in length and 0.77 mm in width. The mandibles of the third instar larva are similar to those of the first instar but larger and measuring 0.081 mm in length and 0.048 mm in width at the base (figure 1H). The spiracles are fully developed and nine pairs are visible from the second to the tenth segment. Tracheal trunks are well formed.

3.1c Pupa: The pupa is brown in colour. The appendages adhere firmly to the body. The average length of 12 pupae was 2.38 mm and the width at the thoracic region was 0.76 mm.

3.2 Life history

T. pantnagarensis is a solitary endoparasite. The egg is laid in the abdomen of the pupa of the primary parasite. The incubation period of eggs ($n=20$) ranged from 42–56 h.

The larval duration for the 3 instars averaged 4.22 days (range 4–5 days). The size of the larva increases gradually from hatching and it entirely fills the host cocoon. When fully grown, no trace of the host pupa remains.

The pupal period recorded for 15 pupae averaged 5.82 days (range 5–6 days). The total life cycle from egg to adult emergence studied for 68 cocoon masses ranged from 11–14 days (average 12.18).

3.3 Adult

3.3a Oviposition: Mating occurs soon after emergence and lasts 20–30 s. Oviposition commences after a preoviposition period of 1–2 days. The duration of oviposition was recorded; females continue to lay eggs for about 7–10 days. Only

one egg is laid in each host even if the host cocoons were repeatedly exposed to the hyperparasite female. It is likely that a host marking mechanism by the ovipositing female prevents superparasitism. The host during the act of oviposition is paralysed and further development of the host is completely arrested.

3.3b Longevity: Newly emerged females when kept in tubes and fed on 20% honey solution live 11.41 days with a maximum of 16 days. The average longevity of males on the same food was 8.51 days. Without food, the adults live for 1–3 days.

Acknowledgement

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Tributyltin oxide induced alterations in exuvial weight and calcium content of the prawn, *Caridina rajadhari*

R NAGABHUSHANAM, P S REDDY and R SAROJINI

Department of Zoology, Marathwada University, Aurangabad 431 004, India

MS received 12 February 1990

Abstract. Moulting is the predominant feature of crustaceans. Cyclic cuticle deposition and resorption of calcium from old cuticle occur in relation to the crustacean moult cycle. Prolonged exposure of the prawn, *Caridina rajadhari* to media containing sublethal doses (0.015, 0.020, 0.025 and 0.040 ppm) of tributyltin oxide led to an apparent increase in dry weight of exuvia as well as an increase in the total quantity of calcium. The increase in weight and calcium content of exuvia was not significant in the initial two moults whereas it was significant ($P < 0.05$) in the third moult when exposed to minimum (0.015 ppm) dose. However significant ($P < 0.01$) rise in both exuvial weight and calcium quantity in the first two moults and highly significant ($P < 0.001$) in the third moult exuvia were observed when exposed to maximum (0.040 ppm) dose. The observed changes in exuvia from prawns exposed to tributyltin oxide might be due to a decrease in the resorption of the old cuticle and/or due to the inhibition of chitinolytic enzymes.

Keywords. Tributyltin oxide; exuvial weight; calcium; elevation; *Caridina rajadhari*.

1. Introduction

Moulting forms the most characteristic and important metabolic event in the life cycle of crustacean animals (Highnam and Hill 1979). Cyclical cuticle deposition and resorption of calcium from old cuticle occur in relation to the crustacean moult cycle (Passano 1960). Though there has been a great deal of research on the impact of aquatic pollutants on moult cycle there have been very few studies to investigate the alterations in the exuvium (cast exoskeleton). Branon and Concklin (1978) studied the effect of sodium pentachlorophenate (NaPcP) on exoskeletal calcium in the grass shrimp, *Palaemonetes pugio*.

The above literature revealed that no attempt has been made on the impact of organotin antifouling compounds, which are extensively used in paint, agriculture and paper industries as a protective biocide. In this paper an attempt has been made to study the impact of sublethal concentrations of tributyltin oxide (TBTO) on exoskeletal weight and calcium on 3 successive moults of *Caridina rajadhari*.

2. Materials and methods

The freshwater prawns, *C. rajadhari* were procured from Kham river near Aurangabad and maintained in plastic troughs having sufficient amount of well aerated tap water. Water was changed daily and the animals were fed with green algae (*Chara*, *Pyranimonas*) twice in a week, and were acclimated to the laboratory conditions for a week prior to the commencement of the experiment.

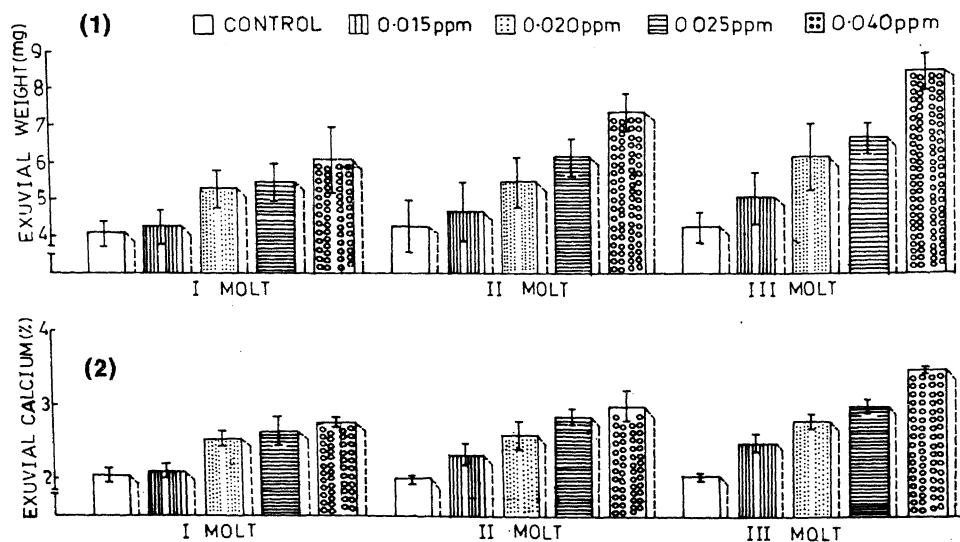
To determine the impact of sublethal concentrations of TBTO on exuvial weight and calcium quantity, prolonged experiments up to the completion of 3 moults in

the test as well as control media were performed. The solutions were not aerated but renewed every 24 h to provide fresh oxygenated media as well as to remove accumulated waste products. Healthy, mature, intermoult (stage C) laboratory acclimated prawns of same size (25 ± 3 mm length from rostrum to telson) and sex (female) were divided into 5 groups of 20 each, among which one group served as control and the remaining 4 groups exposed to 0.015, 0.020, 0.025 and 0.040 ppm of TBTO in 500 ml beakers individually. The jars were examined daily for the cast exuvia.

Cast exuvia were collected following ecdysis and preserved in individual vials after rinsing with deionized, glass distilled water. Precaution was taken not to include exuvia which had been partially eaten by molted shrimp. The exuvia were dried at 110°C for 48 h after which dry weights was measured. After obtaining dry weights, exuvia were processed for calcium analysis as described by Hawk *et al* (1965) with a little modified method by Sarojini *et al* (1983). The calcium content is expressed in percentage.

3. Results

Alterations in exuvial weight and calcium quantity of *C. rajadhari* are shown in figures 1 and 2. The observed data showed that control prawns did not exhibit any change in weight and calcium of exuvia in the 3 moults, whereas it was altered when introduced to TBTO contaminated media. The increase in dry weight and calcium content of exuvia was not significant in the initial two moults, when exposed to minimum (0.015 ppm) dose whereas it was highly significant ($P < 0.01$) when it was exposed to maximum (0.040 ppm) dose. Even the exuvia from prawns exposed to 0.015 ppm TBTO, which underwent third ecdysis were heavier and had significantly greater ($P < 0.05$) weight and higher amount of calcium than control.



Figures 1 and 2. Exuvial weight (1) and calcium content (2) of 3 consecutive moults of the prawn, *C. rajadhari* exposed to TBTO.

Thus the elevation in both weight and calcium content of exoskeleton is dose and exposure period dependent.

4. Discussion

In the present investigation increase in dry weight and calcium quantity of exuvia of prawn administered with different sublethal concentrations of TBTO from initial moult to 3 subsequent moults was noticed. A decrease in the resorption from the old exoskeleton after exposure prior to moult may be one of the reasons for the shedding of heavier exoskeleton. Similar observations and suggestions were reported by Branon and Concklin (1978) in the prawn, *P. pugio* exposed to NaPcP. Sarojini *et al* (1983) observed increase in weight and calcium quantity of exuvia of *C. rajadhari* after exposure to NaPcP and suggested that NaPcP might be preventing the resorption of calcium from the old exoskeleton. These results were also supported by the findings of Sarojini and Reddy (1984), who reported rise in exuvial weight of *M. kistnensis* exposed to sevimol.

Further causative factor for rise in calcium and weight of the exuvia might be due to the activity of chitinolytic enzymes. The passing of minerals from the exuvial space is via the already intercalated new epi- and exocuticle via the epidermis and the basement membrane into the haemolymph. Presumably controlled by the moult hormone, an ecdysteroid (Buchholz 1989) chitinase activity rises sharply at the onset of the premolt (D_0 stage). This coincides with the opening of the exuvial cleft and an increase of glucosamine in the haemolymph may be responsible for apolysis and the initiation of cuticular material resorption (Buchholz and Buchholz 1988). The activity of N-acetyl- β -D-glucosaminase was high when the endoenzyme chitinase reaches its maximum in late precdysis (stage D_3 - D_4) (Buchholz 1989). The relatively high chitinase activity in D_1 - D_2 stage in Antarctic krill, *Euphausia ruperba* and maximum glucosamine concentration in the haemolymph during D_3 - D_4 stage indicates some enzymatic digestion of the old cuticle (Buchholz and Buchholz 1989). For the rapid decomposition of cuticular chitin in D_3 - D_4 stages the above mentioned processes should be effectively operative without any interruption.

Malley and Chang (1985) reported the inhibition of calcium uptake by aluminium at pH 5.5 by the crayfish, *Orconectes virilis*. Patil (1989) observed the rise in dry weight and calcium content of the exuvia when the prawn, *M. lamerrii* exposed to benzene and suggested that this might be due to the prevention of reabsorption of calcium from the old exoskeleton.

By considering the above reports it can be suggested that TBTO might inhibit the resorption of calcium and or inhibits the chitinolytic enzymes resulting in the shedding of heavier exoskeleton with higher quantity of calcium. Further research is necessary to show in which way TBTO acts in the processes of exoskeleton digestion and resorption.

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Protein requirement of juvenile *Penaeus indicus*. 1. Food consumption and growth

C GOPAL* and R PAUL RAJ**

Central Marine Fisheries Research Institute, Cochin 682 031, India

*Present address: Central Institute of Brackishwater Aquaculture, 12 Leith Castle Street, Santhome, Madras 600 028, India

**Madras Research Centre of Central Marine Fisheries Research Institute, 141 Marshall's Road, Madras 600 008, India

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Abstract. Juveniles of *Penaeus indicus* were fed with different concentrations of protein diet (0–60% of dry weight of diet) with purified lipid free casein as protein source. During the experimental period of 30 days, survival was only 27% in the group fed with protein free diet. Survival (75%) and protein efficiency ratio (3.7) were maximum at 40 and 30% protein level, respectively; specific food consumption (4.3–6.7%) and food conversion ratio (1.3–1.4) were lowest at 30 and 40% protein level, respectively. Another experiment conducted to precisely quantify the near optimum protein requirement for maximum growth of *Penaeus indicus* revealed significantly higher growth and protein efficiency ratio in the groups receiving 35–37.5% protein than the other groups.

Keywords. *Penaeus indicus*; juvenile; protein requirement; food consumption; growth; protein efficiency.

1. Introduction

Protein requirement of prawns has been well studied and reviewed from time to time by a number of workers (New 1976; Maguire 1980; Pandian 1989). It is known to range from 22–60% of dry weight of the diet (Shewbart *et al* 1972; Deshimaru and Yone 1978). The wide range of values on protein requirement is mainly due to difference in the size of the experimental prawns, physiological status and other intrinsic and extrinsic factors (Abdel Rahman *et al* 1979; Maguire 1980). However, available studies on post-larval or juvenile *Penaeus indicus* suggests that the optimum protein requirement ranges from 40–43% of dry weight of the diet (Colvin 1976; Ali 1982; Bhaskar and Ali 1984). Though indicative of near precise quantification of protein requirement of *P. indicus*, these studies were conducted by offering qualitatively none too superior diet to the post-larvae juveniles. These studies were conducted by using semi-purified or compounded diets and only limited studies are available on the protein requirement of prawns by using purified diets (Bhaskar and Ali 1984). As purified diets considerably reduce extraneous nutritional factors and allow precise quantification of any particular nutrient requirement (D'Abramo *et al* 1982), more information is required on the protein requirement of prawns by using purified diets. In the present study, the following two experiments were conducted on the juveniles of *P. indicus* by using casein (lipid free), which is the only protein source available in highly purified state: (i) the effect of different protein levels (0–60% of dry weight of diet) on growth and food consumption, and (ii) near optimal quantification of protein in the juveniles by using test diets ranging from 32.5–47.5% of protein.

2. Materials and methods

2.1 *Experimental animals*

Juveniles of *P. indicus* belonging to the same brood stock were transported from Narrakal Prawn Hatchery of CMFRI, Cochin to the laboratory. The post-larvae were reared under laboratory conditions for 15–20 days and fed with compounded diets until they reached the required size (length: 20 ± 5 mm; live weight: 20 ± 8 mg). Healthy juveniles of uniform size were blotted in the folds of filter paper, weighed to the nearest mg and transferred to the experimental aquaria. In each aquarium, 20 juveniles were reared and each treatment had 3 replicates. Prior to the commencement of the experiment, the experimental animals were starved for 24 h and allowed to recover from handling stress. To determine the initial dry weight, a few prawns were initially weighed and sacrificed by immersing in boiling water for a brief period (Clifford and Bricks 1983) and left for drying at 40°C for 48 h in an oven. The dried samples were reweighed. Similar procedure was adopted at the end of the experiment to determine the final dry weight of the prawns. Growth of the prawns was calculated and represented as percentage increase on wet or dry weight basis.

2.2 *Experimental aquaria*

The experiments were conducted in plastic aquaria (54 cm dia.; 24 cm depth), mounted on vertical steel racks. In each aquarium 40 l of filtered, irradiated and dilute seawater (salinity: $20 \pm 2.5\text{‰}$) (Colvin 1976) was used. The water was continuously aerated and was replaced on alternate days.

2.3 *Test diets*

Iso calorically adjusted graded levels of protein test diets from 0–60% with an interval of 10% protein for the first experiment and from 32.5–47.5% with an interval of 2.5% protein for the second experiment were formulated following earlier studies (Kanazawa *et al* 1970, 1977; Adelung and Ponat 1977; Conklin *et al* 1978). Finely powdered, preweighed ingredients except cellulose, starch, lipids (oils) and vitamins were mixed in Warring blender. Gelatin was dissolved in cold water and later boiled with cellulose and starch. After gelatinization, corn oil and cod-liver oil containing fat soluble vitamins were added and heated in water bath for 10 min. The ingredients were mixed thoroughly and then steamed at 115 lb pressure for 5 min. The steamed feed was allowed to cool to room temperature and then the vitamin mixture was added and mixed thoroughly. The pH of the diet was adjusted to 6.8 (Kanazawa *et al* 1977) using 0.1 N NaOH and the feed was stored in polyethylene bags in a freezer. The moisture content in the feed was adjusted to 30%. Before feeding the prawns, the feed was thawed to room temperature and made into small balls and weighed. The protein content in the diets were accordingly adjusted without changing the calorie content. The composition of the formulated diets for each experiment is as shown in table 1.

Table 1. Composition of experimental diets.

Ingredient g/100g	Experiment 1										Experiment 2						
	0	10	20	30	40	50	60	32.5	35	37.5	40	42.5	45	47.5			
Casein (lipid-free)	—	11	21	31	41	51	61	34	36	39	41	44	46	49			
Egg albumin	—	1	1	1	1	1	1	1	1	1	1	1	1	1			
Gelatin	—	1	1	1	1	1	1	1	1	1	1	1	1	1			
Glucosamine-HCl	—	1	1	1	1	1	1	1	1	1	1	1	1	1			
Sucrose	20.11	19.9	15.43	11.68	7.24	4.68	1.18	10.25	9.65	8.28	7.24	6.86	6.65	5.45			
Glucose	10.19	7.19	6.53	4.96	3.68	2.32	0.9	4.61	4.41	4.20	3.68	3.33	2.75	2.5			
Starch	43.45	32.59	27.39	22.61	18.51	12.98	7.65	21.65	20.58	18.89	18.51	15.61	14.92	13.66			
Cod-liver oil	6	6	6	6	6	6	6	6	6	6	6	6	6	6			
Corn oil	3	3	3	3	3	3	3	3	3	3	3	3	3	3			
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5			
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3			
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3			
Mineral mixture*	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41			
Vitamin mixture**	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24			
Agar-agar	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Cellulose	4	4	4	4	4	4	4	4	4	4	4	4	4	4			

*CaHPO₄·2H₂O 2.5; MgSO₄·7H₂O 2.0; KH₂PO₄ 1.5; NaH₂PO₄·2H₂O 1.0; MnSO₄·H₂O 0.14; FeSO₄ 0.1; ZnSO₄·7H₂O 0.1; C₆H₅O₇·Fe·5H₂O 0.05; Co(NO₃)₃ 0.01; CuSO₄·5H₂O 0.01.

**B-Carotene 0.014; calciferol 0.002; α-tocopherol acetate 0.032; menadione 0.032; ascorbic acid 2.424; thiamine hydrochloride 0.01; riboflavin 0.008; nicotinic acid 0.032; pyridoxine hydrochloride 0.016; calcium pantothenate 0.06; folic acid 0.001; p-aminobenzoic acid 0.014; choline chloride 0.3; inositol 0.3; biotin 0.004; cyanocobalamin 0.001.

2.4 Estimation of food consumption

The prawns were fed with the test diets twice a day at the rate of 10% (dry weight feed) of live body weight/day. The uneaten food was collected every day by siphoning the water through bolting silk; the residue was washed in distilled water to remove the adhering salts and transferred to preweighed aluminium foils, dried and weighed at 70°C for 48 h. The total dry feed consumed by the prawns in each group was determined every day by subtracting the dry weight of uneaten food from the dry weight of food offered. Specific food consumption (SFC) (Bordner and Conklin 1981; Gopal 1986), food conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows:

$$\text{SFC (\%)} = \frac{\text{Total initial dry wt. of food offered} - \text{Total final dry wt. of food uneaten}}{\text{Number of animals surviving at the end of experiment} \times \text{experimental period (days)} \times \text{mean animal wet wt.(g)}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry wt. of food offered} - \text{Total dry wt. of uneaten food}}{\text{Final wet wt. of prawns} + \text{Wet wt. of dead prawns} - \text{Initial wet wt. of prawns}}$$

$$\text{PER} = \frac{\text{Final wet wt. of prawns} - \text{Initial wet wt. of prawns}}{\text{Total protein intake}}$$

For each of these parameters, the mean values of the replicates per treatment was considered for the statistical calculation.

2.5 Experimental conditions

The mean water temperature, pH and ammonia levels in the aquaria for both experiments were $27.7 \pm 1.9/27.6 \pm 2.27^\circ\text{C}$; $8.4 \pm 1.0/8.02 \pm 0.5$ and $0.04 \pm 0.01/0.02 \pm 0.003$ $\text{NH}_4\text{-N}$ mg/l/day, respectively. Samples of each diet were analysed for crude protein, lipids and moisture following the methods suggested by AOAC (1975). Water samples were analysed using standard methods (Strickland and Parsons 1972; Spotte 1979).

2.6 Statistical analysis

All the parameters (SFC, FCR, PER) estimated are apparent and no correction factor was introduced for the exuviae and dead prawns eaten by the cohabitators during the experimental study. The data collected were statistically analysed using the test of significance and variance (ANOVA) and the means of the treatments were compared by the least significance difference method (LSD) (Snedecor and Cochran 1973).

3. Results and discussion

3.1 Survival

The protein concentration in the diet significantly ($P < 0.05$) influenced the survival of *P. indicus*. During the experimental study of 30 days, only 27% of prawns receiving 0% protein level survived and the percentage of survival was 75% in the group receiving 40% protein (figure 1). As more than 50% of prawns survived at 10% protein level, 10% protein in the diet may be considered as the critical protein level for the survival of the juveniles. Beyond 40% protein, the survival rate declined, indicating the adverse effect of excess dietary protein on the prawns. Weekly survival rate at different protein levels shows that the mortality of prawns fed with protein free diet was higher from the second week onwards. It was observed that most of the prawns receiving protein free diet succumbed immediately after molting. They were also not active and the cohabitators resorted to cannibalism. Cannibalism was evident in all the groups, more so in the groups receiving excess protein ($> 40\%$).

3.2 Specific food consumption

The SFC of *P. indicus* was influenced by the protein content in the diet. It was 33.7% for the groups receiving protein free diet as against amongst the groups receiving 10–60% protein, it was minimum (4.3%) at 40% protein (figure 2). Thus, prawns fed on protein free diet consumed 8 times more than the juveniles fed with protein in the diet. In spite of the fact that the prawns fed with protein free diet consumed more than the other prawns, even then there was large scale mortality suggesting that protein forms the basic nutrient in the diet of the prawns. Further it was observed that as the protein concentration increases, the SFC falls sharply up to 40% protein level. Thus, it appears that juveniles of prawns when exposed to

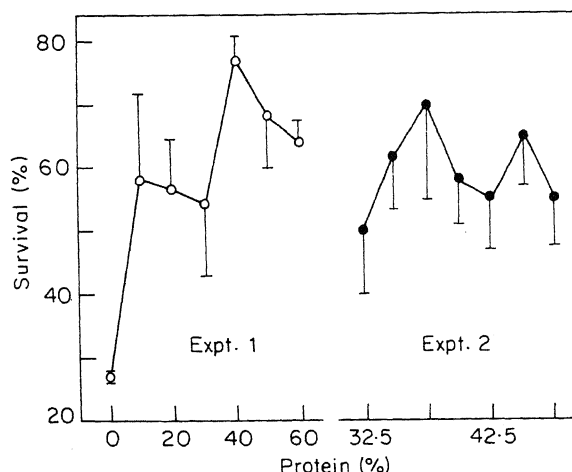


Figure 1. Per cent survival of juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32.5–47.5%).

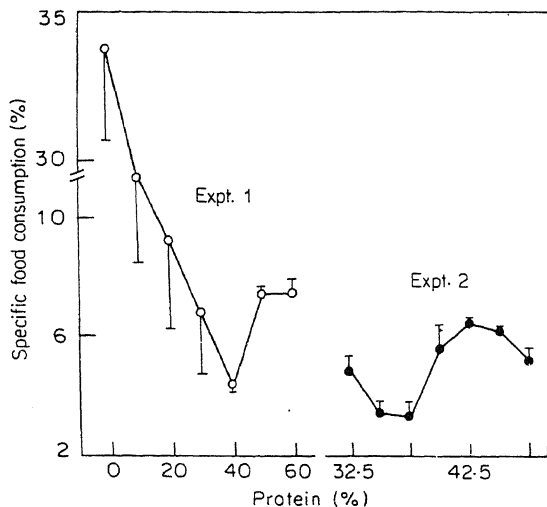


Figure 2. Specific food consumption in juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32.5–47.5%).

optimum level do not load the stomach with excess energy but prefer to consume required quantum of protein based energy. However, exposure to very high protein levels (50 and 60%) induces the prawns to increase the food consumption, thereby loading the stomach with very high protein energy.

3.3 FCR and PER

The FCR and PER (figure 3) were also significantly influenced by the protein content in the diet. The FCR sharply declined from 4.6 ± 0.4 in the prawns fed with protein-free diet to 1.3 ± 0.9 at 30% protein; these values were statistically significant ($t=16.9$; $P<0.05$). Similarly, the prawns fed with 30% protein had significantly higher PER than the PER of other groups.

3.4 Growth

The mean gain in dry weight of the prawns increased from 125% in the group exposed to protein-free diet to 522% in the group exposed to 40% protein and thereafter declined to 430% at 60% protein (figure 4). The sharp increase in growth of the group receiving 30% protein than the earlier group (20% protein) and the maximum growth at 40% protein in terms of dry as well as wet weight suggest that 30–40% of protein may be the optimum level for the maximum growth of the juvenile prawn. The difference in mean gain in dry weight between 30 and 40% protein levels was not statistically significant (dry wt.; $t=0.25$, $P>0.05$). The decline in growth at high protein levels (50 and 60%) reveals the deleterious effect of excess protein in the diet (Venkatramiah *et al* 1975; Bages and Sloane 1981; Vernberg 1987). Thus, it was observed that when juvenile prawns are fed with optimum protein diets, the growth and protein efficiency tends to be maximum and the

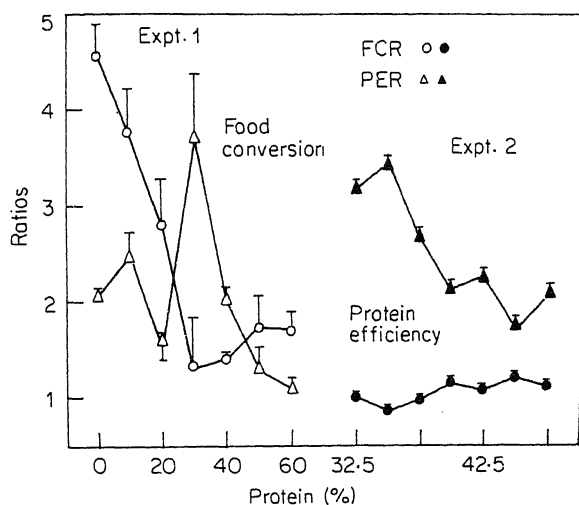


Figure 3. Food conversion ratio and protein efficiency ratio in juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32.5–47.5%).

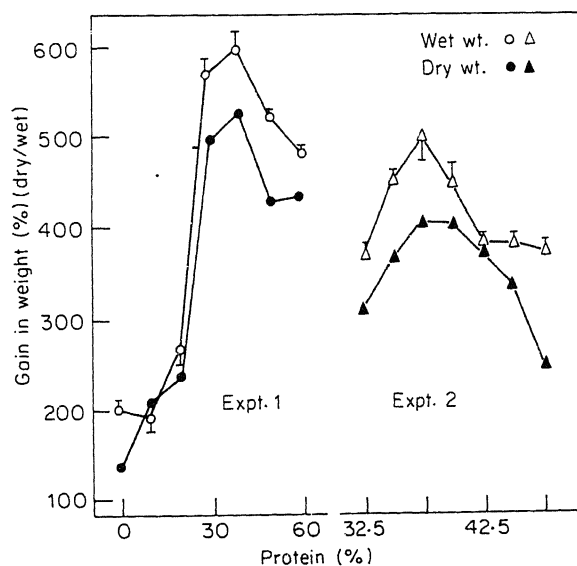


Figure 4. Per cent gain in weight by juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32.5–47.5%).

specific food consumption and food conversion ratio are the lowest thereby suggesting the importance of protein based energy diets which are efficiently utilized by the animals.

3.5 Near optimal protein requirement

The second experiment on the juveniles of *P. indicus* was conducted based on the

results of the first experiment to precisely quantify the optimal protein requirement. It was observed that there was no significant difference in the survival percentage in the test groups (32.5% vs 47.5%; $t = 1.35$; $P > 0.05$) since the diets offered were near optimal requirement. However, comparing other parameters it was observed that the SFC was significantly low at 35% (3.4%) and 37.5% (3.3%) protein levels compared to the SFC of other protein levels (figure 2). For instance, the SFC of juveniles feeding on 37.5% protein diet ($3.3 \pm 0.6\%$) was significantly ($t = 20.42$; $P < 0.05$) lower than the SFC of juveniles feeding on 40% protein diet ($5.6 \pm 0.7\%$). Similarly, the FCR (0.8) was the lowest and the PER (3.5) was the highest at 35% protein level. Thus, all these parameters substantially augment to the maximum mean gain in weight obtained at 37.5% protein level thereby indicating the optimal level around 35 and 37.5% protein level (figure 4). The growth of the juveniles declined beyond 40% protein, confirming the growth pattern obtained in experiment 1 (figure 4).

A comparison of food consumption and growth of the prawns of the experiments 1 and 2 revealed a few differences between these two experiments. The growth, especially, was slower in experiments 1 and 2. The difference may be due to genetic variations in the brood stock, as the juvenile prawns used for the experiments were from two different stocks. Nevertheless, a definite trend was obtained in experiment 2 which has facilitated identification of near optimal protein requirement of the juvenile prawns.

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Effect of heavy metal salts on the life history of *Daphnia similis* Claus (Crustacea: Cladocera)

SUDHA SOUNDRAPANDIAN and K VENKATARAMAN*

School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625 021, India

*Zoological Survey of India, Port Blair 744 101, India

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Abstract. The acute toxicity of heavy metal salts on *Daphnia similis* Claus is studied in terms of LC_{50} . Copper is found to be the most toxic and zinc the least. Toxicity of the heavy metals is studied by observing changes in the longevity, body length, fecundity and moulting frequency of the animal. The decreasing order of toxicity of heavy metals on the longevity, body length and fecundity is indicated by $Zn > Pb > Cu > Hg$, $Cu > Zn > Hg > Pb$ and $Pb > Cu > Zn > Hg$ respectively. The average number of instars increased with the increase of the concentration of metals in the medium. The order of accumulation pattern is $Zn > Pb > Cu$ for 24 h and $Zn > Cu > Pb$ for 48 h. On doubling the concentration of metal to which *Daphnia similis* is exposed and fed to the fish *Sarotherodon mossambicus*, the biomagnification increases for copper and zinc while it decreases for lead.

Keywords. Heavy metal salts; acute and chronic studies; *Daphnia similis*.

1. Introduction

As most waters from industrial wastes that contain heavy metals are released into continental waters, studies on the effect of these metals on the aquatic life have in recent years attracted the attention of many ecologists. Cladocerans constitute one of the dominant zooplankton of the aquatic ecosystems experimenting varied polluted conditions. Some of them exist even in highly polluted waters serving as indicators of water pollution. Various species of *Daphnia* have been used as test organisms to study the effect of heavy metals in temperate regions, while the information is lacking in tropics (Eaton 1973; Winner and Farrel 1976; Leeuwangh 1977; Chen *et al* 1980; Winner 1981). As *Daphnia* forms an important source of food for many aquatic organisms, it has been used to study the effect of heavy metals through food chain. However, the metal content in this crustacean after exposure is not known. The present study is intended to ascertain the effect of various sublethal concentrations of some heavy metal salts on the life of *Daphnia similis* Claus, their accumulation pattern and biomagnification when they are used as food.

2. Materials and methods

Heavy metal solutions were prepared using $CuSO_4 \cdot 5H_2O$; $Pb(CH_3COO)_2 \cdot 2H_2O$; $HgCl_2$ and $ZnSO_4 \cdot 7H_2O$ salts, the concentrations being 0.1, 0.05, 0.01, 0.005 and 0.001 ppm. The test organisms of *D. similis* were collected from an artificial tank set under laboratory conditions (pH 8.15 and DO 2 ppm) and the animals fed with organic matter from the cow dung slurry from a biogas plant. LC_{50} fresh samples of adult parthenogenetic females of *D. similis* are maintained in 40 ml of each of the

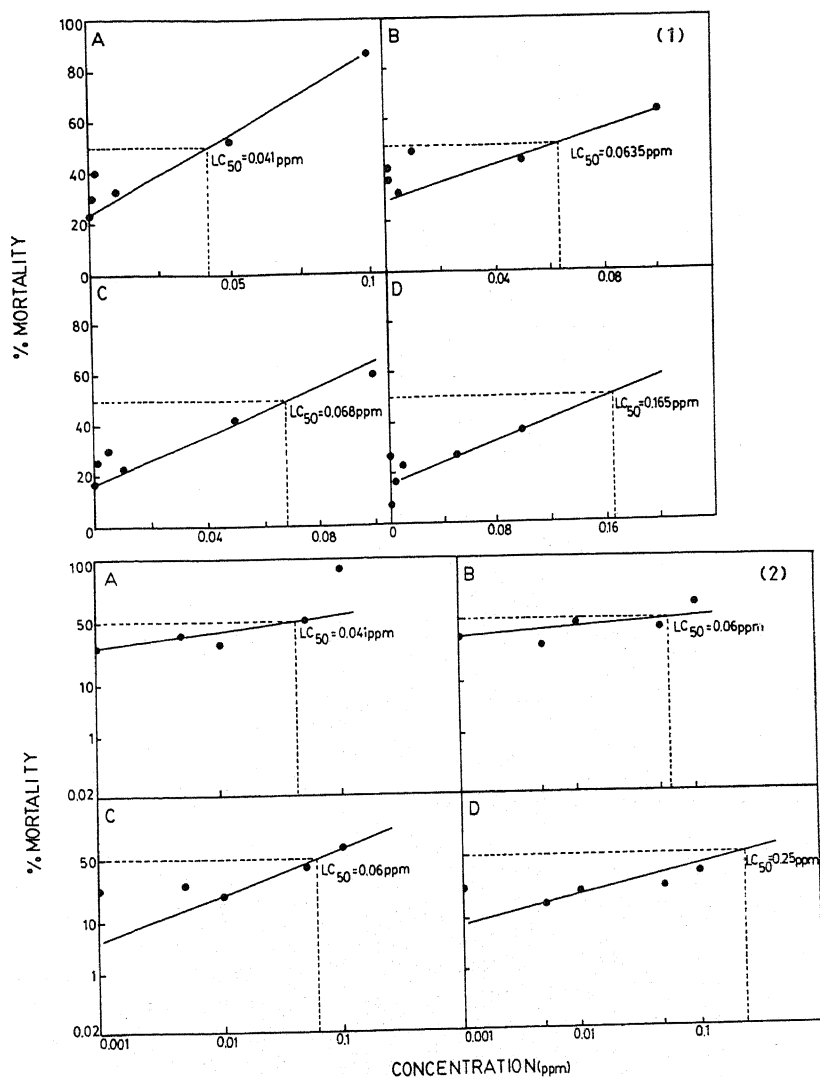
heavy metal solutions and examined daily for mortality during a period of 96 h. LC_{50} values were calculated by graphical and probit-graph methods. Tests were conducted in triplicate under laboratory conditions (28° – 30° C).

Each sublethal toxicity test was initiated with 10 samples of *D. similis* (approximately 24 h old) reared in 40 ml of metal salt solutions of various concentrations prepared in tank water. Animals were changed every day to freshly prepared metal concentrations and the young ones observed through a compound microscope. Mortality and reproductive activities were determined. The number of instars and the young ones produced in each instar were noted (Venkataraman 1981). Body length was measured using an ocular micrometer. Mean body lengths and brood sizes were compared among metal concentrations by analysis of variance. Longevities were compared by percentage survivors.

A known amount of adult *D. similis* was exposed to selected metal concentrations in triplicate for 24 and 48 h. The material was filtered, dried and weighed to the nearest 0.001 mg to study bio-accumulation. It was later digested in 0.5 ml of nitric acid at 60° C and diluted to 6 ml. The metal concentrations were determined by Perkin Elmer 372 atomic absorption spectrophotometer. Metal loads were expressed as bioconcentration factor (BCF) mg metal g^{-1} daphnid/ mg metal g^{-1} water. The concentration of copper, lead and zinc in *D. similis* before exposure was 0.02, 0.06 and 0.05 ppm, respectively. Adult daphnids exposed to metal concentrations in triplicate for 24 and 48 h were filtered, rinsed with water and fed to a fish fry of *Sarotherodon mossambicus* (approximately 2 cm). The fish was dried, powdered and digested in a triplicate acid medium. The digest was diluted and analysed for metal load by atomic absorption spectrophotometer. Biomagnification has been expressed as mg metal g^{-1} fish/ mg metal g^{-1} daphnid.

3. Results

The 96 h LC_{50} values obtained by graphic and probit methods are shown in figures 1 and 2. The concentration at which 50% mortality occurs is highest for zinc and lowest for copper, the decreasing order of their sensitivity to heavy metals being $\text{Cu} > \text{Pb} > \text{Hg} > \text{Zn}$. The longevity of *D. similis* exposed to different concentrations of metals is estimated and expressed as the mean duration of life. The stress of mercury became very pronounced only in 0.05 and 0.1 ppm, while animals exposed to copper, lead and zinc suffered a severe decrease in longevity, almost equally in all concentrations (figure 3). The order of decreasing toxicity of heavy metals on longevity of *D. similis* is $\text{Zn} > \text{Pb} > \text{Cu} > \text{Hg}$. The effect of heavy metals assessed on the growth of *D. similis* is shown graphically (figure 4) and by analysis of variance. The variation in growth in lead solutions is not significant, while in all other metal solutions it exhibited statistically significant results (table 1). The decreasing order of toxicity of heavy metals on the growth of *D. similis* is $\text{Cu} > \text{Zn} > \text{Hg} > \text{Pb}$. Compared to other metals, the mercury stress on fecundity is less prominent (figure 5). Considering the average number of eggs laid by an animal per day, the decreasing order of toxicity of heavy metals on egg production is $\text{Pb} > \text{Cu} > \text{Zn} > \text{Hg}$ (table 1). For any specific concentration, the order of bioaccumulation is $\text{Zn} > \text{Pb} > \text{Cu}$ during 24 h and $\text{Zn} > \text{Cu} > \text{Pb}$ during 48 h exposure (table 2). On doubling the concentration of metal to which *D. similis* is exposed and fed to the fish, the biomagnification increased for copper and zinc, while it decreased for lead



Figures 1 and 2. Acute toxicity of (A) copper, (B) lead, (C) mercury and (D) zinc salts on *D. similis*; 96 h LC₅₀ determined by (1) graphical and (2) probit-graph methods.

in the order of Pb > Cu > Zn in 0.05 ppm and Cu > Zn > Pb in 0.1 ppm concentration (table 3). The average number of instars increased with increasing concentration of heavy metals than in the control medium (table 4).

4. Discussion

Chronic effects of metals may be detectable more quickly with smaller species of *Daphnia* which have shorter life spans (Winner and Farrell 1976). The significant reduction in longevity in lead, zinc and copper solutions in the present study confirms *D. similis* as a smaller sized species with shorter span of life showing more

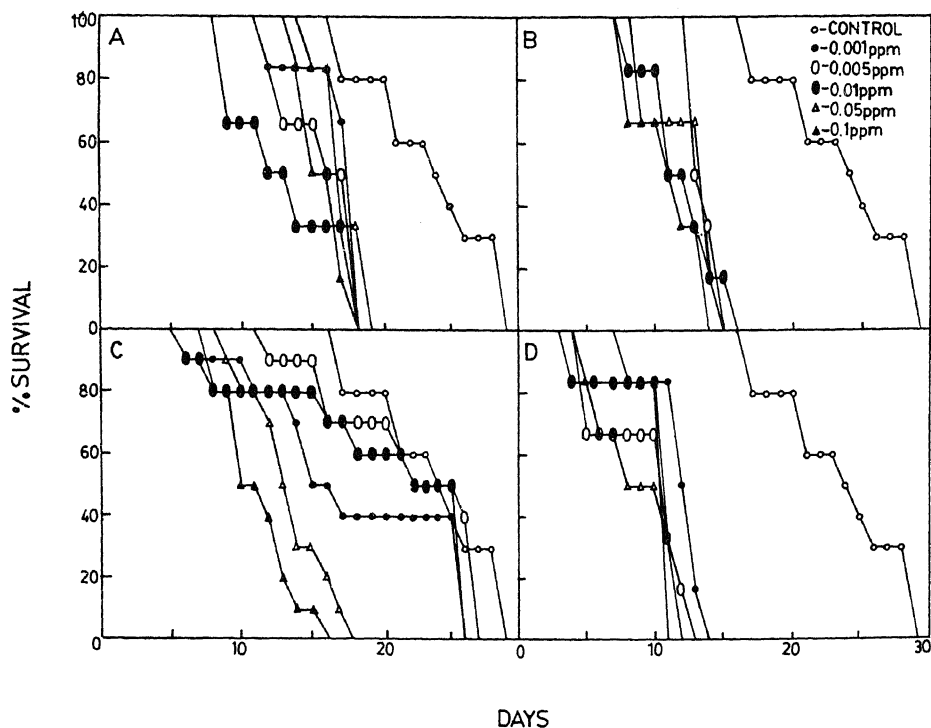


Figure 3. Survivorship curve of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

detectable chronic effects. However, mercury does not decrease the longevity of *D. similis* to a large extent at lower concentrations probably due to its use in inorganic form.

For all the 4 metals investigated, concentration above 0.005 ppm causes reduction in body length of *D. similis*. Reduction in body size of *D. magna* has also been observed due to copper and zinc stresses (Winner 1981). The growth curves showed a steep rise in the initial stages, while it was gradual after the attainment of primiparous stage. Thus, there does not seem to exist any distinct relationship between concentration and growth (figure 4). The stress of heavy metals was reflected in the average number of instars per day. In 0.1 and 0.05 ppm, the number of instars were more than in any other concentration showing an increased number of instars (table 4). The average number of instars in the present study increased with the increasing concentration, which may facilitate the excretion of metal load as suggested by Dethlefsen (1978) and Bertram (1980).

Reproductive impairment due to copper stress have earlier been observed in *D. magna* (Dave 1984), *D. pulex* (Ingersoll and Winner 1982), the present study also confirming the copper concentration taken for the life history study resulting in decreased brood size (figure 5). As the administration of mercury was in the form of mercuric chloride, the accumulation of mercury in *D. similis* was perhaps less than that of other metals and hence their lower reduction in brood size and longevity. The demonstration of trophic transfer of mercury in the form of mercuric chloride from *C. vulgaris* to *D. magna* supports this view (Ribeyre 1981). Hall (1964) has

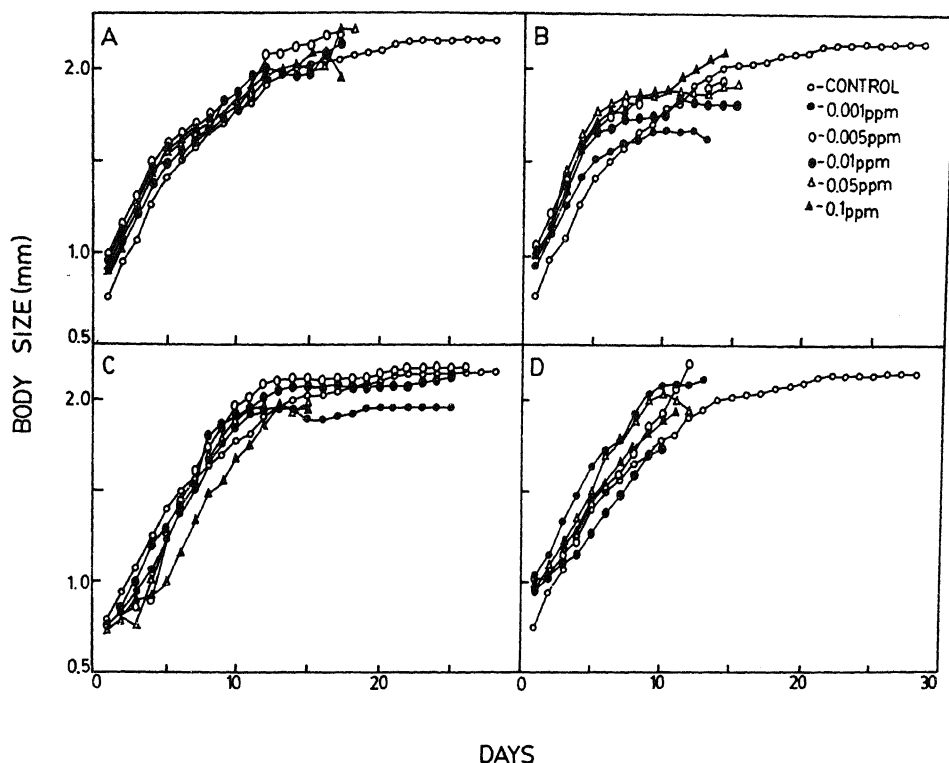


Figure 4. Growth curve of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

Table 1. Differences in growth and fecundity of *D. similis* in relation to number of days, reared in 5 different concentrations of 4 different metals and in a control medium (ANOVA summary table).

Heavy metal	F value		df		P	
	Growth	Fecundity	Growth	Fecundity	Growth	Fecundity
Copper	5.998	11.170	5, 118	5, 108	0.001**	0.01**
Lead	2.050	9.850	5, 94	5, 93	0.1*	0.01**
Mercury	2.810	2.123	5, 128	5, 128	0.05**	0.1*
Zinc	3.033	4.760	5, 80	5, 80	0.025**	0.01**

*Not significant; **significant.

shown that brood sizes are determined by the nutritional state of the female during the period of egg development. However in the present study, the temperature and food resources remaining the same for all animals, the differences in reproductive performance were probably due to metal stress.

In 0.1 ppm concentrations of all the metals, the bioconcentration factor exhibited an increase when the duration of exposure was increased from 24–48 h. However, with 0.05 ppm concentration, the bioconcentration factor decreased with increase in exposure time. No such regular trend was noticed for other concentrations. Among

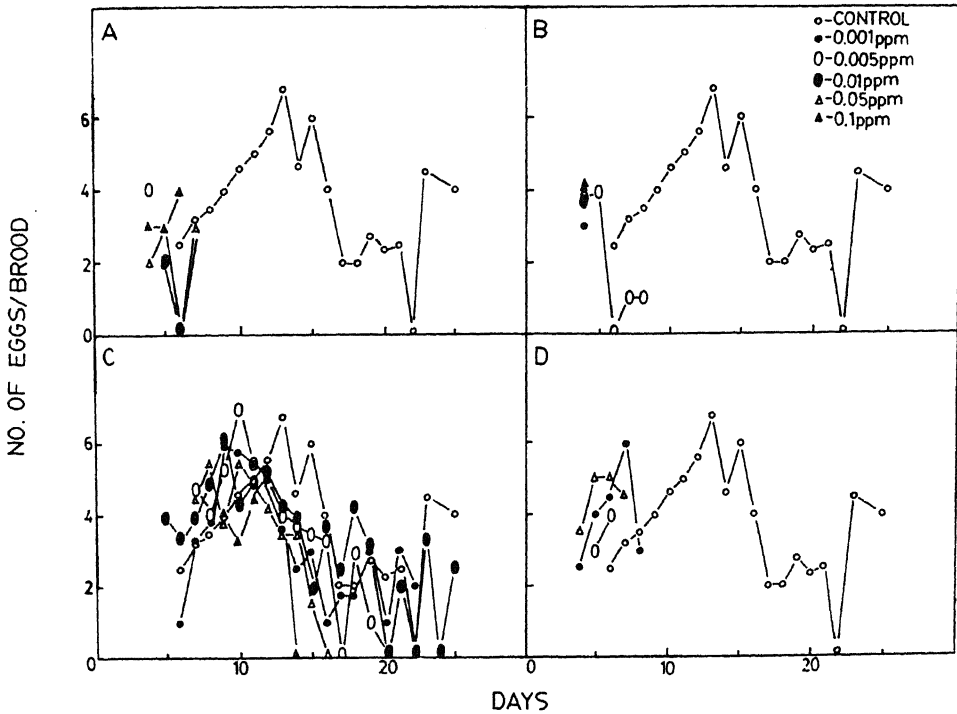


Figure 5. Fecundity of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

Table 2. Bioaccumulation of heavy metal in *D. similis* after 24 and 48 h exposure.

Heavy metal	Conc. of metal in water (ppm)	Bioconcentration factor	
		24 h	48 h
Copper	0.001	4.1150	10.9900
	0.005	0.7338	0.5128
	0.01	0.2353	0.3333
	0.05	0.0741	Not read
	0.1	0.0330	0.0265
Lead	0.001	4.9260	5.5560
	0.005	0.8602	0.3243
	0.01	0.2500	0.4142
	0.05	0.0804	0.0860
	0.1	0.0513	0.2564
Zinc	0.001	24.7600	30.5600
	0.005	2.3000	2.0000
	0.01	2.2500	1.7200
	0.05	1.0000	0.3412
	0.1	0.1520	0.2037

solutions of the same metal, the bioconcentration factor decreased with increase in concentration. Bioaccumulation of copper by daphnids may also be affected by water hardness, humic acid concentration, age at which the animals were exposed

Table 3. Biomagnification of heavy metals in the fish *S. mossambicus* fed with *D. similis* exposed to heavy metals for a duration of 24 h.

Heavy metal	Conc. in water (ppm)	Conc. of metal in fish/wt. of fish	Conc. of metal/wt. of <i>D. similis</i>	Biomagnification
Copper	0.05	1.3344	3.704	0.36
	0.1	1.7544	3.297	0.53
Lead	0.05	4.3230	4.020	1.08
	0.1	2.4096	5.128	0.47
Zinc	0.05	4.7770	50.000	0.10
	0.1	7.9125	15.240	0.52

Table 4. Average number of instars produced by each animal per day when exposed to different concentration of heavy metals.

Heavy metal	Concentration (ppm)				
	0.001	0.005	0.01	0.05	0.1
Copper	0.57	0.66	0.67	0.67	0.72
Lead	0.76	0.75	0.67	0.78	0.78
Mercury	0.69	0.68	0.68	0.80	0.77
Zinc	0.71	0.57	0.68	0.75	0.81

Number of instars per day in control medium = 0.67.

to metals and the total organic carbon present in the medium (Winner 1986). The lower accumulation of copper in *D. similis* in the present study was perhaps due to the nutritionally rich organic medium (biogas spent cowdung slurry) used.

Accumulation of zinc was more than that of copper or lead in *S. mossambicus*. In experiments demonstrated by Vighi (1981), lead accumulated in the trophic chain with a decreasing concentration factor from the lowest to the highest levels confirming with the observations made in the present study.

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Development of visual cells in the toad *Bufo melanostictus* (Schneider)

SULTANA NIAZI and I A NIAZI

Department of Zoology, University of Rajasthan, Jaipur 302 004, India

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Abstract. Differentiation of visual cells in *Bufo melanostictus* begins after hatching at gill bud stage. It is indicated by emergence of cytoplasmic buds from the outer nuclear layer of the retina. Each bud contains an apically located vacuole which soon disappears from all cells. Cones and rods start differentiating simultaneously but the majority of early cells are rods. Single cones, red rods and double cones can be identified by the stage when the operculum is formed. Up to the beginning of hindlimb morphogenesis rods remain the most numerous but later single cones attain approximate numerical equality with rods and the tadpole retina becomes a truly duplex retina. Red rods of tadpoles are structurally different from those of adult. As in the accessory members of double cones a paraboloid develops in the myoid region of the inner segment of red rods also and persists throughout larval life. During metamorphosis green rods appear, cone outer segments become sharply pointed, myoid develops in the red rods replacing the paraboloid which disappears along with its glycogen but there is no change in the double cones. After metamorphosis number and size of red rods increase greatly transforming the duplex retina of the tadpole into a predominantly rod retina adapted for scotopic vision of the nocturnal adult.

Keywords. Amphibia; *Bufo melanostictus*; visual cells; paraboloid.

1. Introduction

The development and structure of eye and its component parts are basically similar in all vertebrates but numerous variations in visual system related to particular modes of life are found in different taxa (Walls 1942). Amphibians include a great variety of species differing widely in habits and photic environment of the habitats during various phases of their life. In some species development is direct and at birth the offsprings are already miniature adults but in most amphibians there is a free living aquatic larval phase followed by metamorphosis of the tadpoles into adult form. In these, development of eye and its parts occurs during the larval and metamorphic periods. While in some species adults are also aquatic, in most others they are terrestrial or amphibious. The larvae are usually diurnal but adults may be diurnal, arhythmic, or nocturnal in visual habits (Blair 1976). Accordingly, many structural and functional variations may be found in the visual system in the course of its development related to the photic and other conditions during the larval and adult phases in different species. Studies on retinal photopigments have vividly demonstrated such differences in some anuran species. In the aquatic tadpoles of several anurans the retinal photopigment is found to consist of a mixture of porphyropsin and rhodopsin among which the former is the dominant pigment. During metamorphosis of ranid tadpoles the proportion of rhodopsin increases, making it the dominant pigment in the retina of adult frogs which are terrestrial or amphibious. *Xenopus*, on the contrary, remains submerged in water in adult stage also and in this anuran proportion of porphyropsin increases further as the tadpole changes into adult form (Reuter *et al* 1971; Crescitelli 1973). However, among the

anurans most investigations on the visual system have been made on a few species of ranid frogs and *Xenopus*. Studies on the development of visual cells during the larval life and metamorphosis to adult stage in a variety of species have been almost completely neglected. The comparative study on the visual cell development in *Rana temporaria* and *Xenopus laevis* by Saxen (1954) still remains the most comprehensive investigation of this kind. In this paper we present observations on the development of visual cells in the toad, *Bufo melanostictus*, from after hatching through larval and metamorphic periods to adult stage. The tadpoles of this toad are aquatic, herbivorous and diurnal and adults are strictly terrestrial, carnivorous and nocturnal. The duplex retina of diurnal tadpoles changes into a predominantly rod retina of nocturnal adults during metamorphosis.

2. Materials and methods

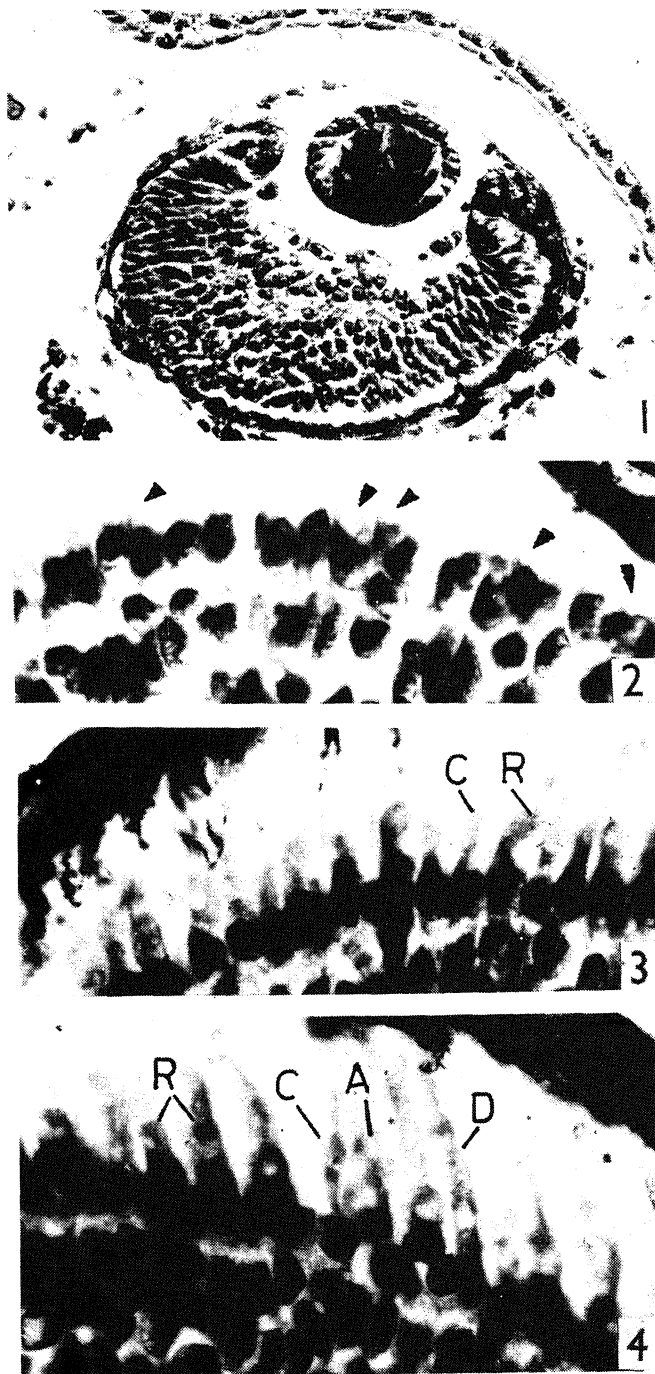
Spawns were obtained from amplexing couples caught from a pond and the tadpoles hatched in the laboratory were reared in well water. They were staged according to the normal table for *B. melanostictus* (Khan 1965). The studies were made on eyes of tadpoles of stages 18 (hatching), 20–21 (gill buds), 24–25 (opercular development), 28–30 (hindlimb buds), 34–38 (well developed tadpoles), 39–42 (metamorphosis), 43 (toadlet) and adults.

Whole heads of young, isolated eyes of older stages and retinal strips of adults were fixed, wax blocked and serially sectioned dorsoventrally at a thickness of 6 μ m. In the case of older tadpoles and toadlets the lens was removed before blocking in wax to avoid damage to retina and the knife during sectioning. Bouin's fluid was used as a fixative and staining with Mallory-Heidenhain's Azan method was found most suitable. For visualization of glycogen the eyes and retinal strips were fixed in ice-cold methacarn (60 ml methyl alcohol + 30 ml chloroform + 10 ml glacial acetic acid) according to Puchtler *et al* (1970) as described in Humason (1972), wax blocked and sections were stained with PAS. Control sections were pre-digested with saliva.

Visual cells are most clearly visualized in sections of dark adapted eyes in which pigment of the pigmented epithelium is retracted exposing these cells. Therefore, many tadpoles of each stage, toadlets and adults were kept in a completely dark chamber for at least 8 h before fixation.

3. Results

On hatching at stage 18 the embryo cannot yet see and the eyes are at a very early stage of development. By this time the optic vesicle has become a double walled cup whose thin outer wall (pigmented epithelium) is still continuous with the neuroepithelium of the brain, the inner wall (sensory retina) is thick, containing many nuclei but without any overt regional or cellular differentiation and the lens placode is still attached to the epidermis. By stage 22 (figure 1) the lens placode has separated, become vesicular and partly filled with fibre cells; the corneal epidermis appears transparent and mesenchymal cells which form the inner cornea have invaded the space between the lens and the corneal epidermis. Beginning of regional differentiation in the sensory retina is now indicated by the appearance of the inner plexiform layer which separates the ganglion cell layer in the central part of the



Figures 1-4. 1. Cross section of eye of a stage 22 tadpole ($\times 125$). 2-4. Visual cells of tadpoles of stage 22 (2), stage 24 (3) and stage 26 (4). Arrow heads indicate achromatic vacuoles in visual cell buds in 2. Note paraboloid precursors in red rods in 3 and 4 ($\times 1000$). (A, Accessory cone; C, single cone; D, double cone; R, red rod).

developing retina. By stage 24 the outer plexiform also appears separating the inner and outer nuclear layers from each other. Ora serrata marks the junction between the peripheral growth zone and the sensory part of the retina; visual cells are better differentiated and the optic nerve now connects the retina with the brain. Further development proceeds gradually as the tadpole grows to its full size and then metamorphoses into adult stage. Differentiation of lens is completed by stage 30 when the vascular choroid and fibrous sclerotic coats also start developing. Iris is well formed by stage 36. Chondrogenesis in the sclerotic coat, fusion of inner and outer corneas and formation of nictitating membrane occur during metamorphosis.

3.1 *Development of visual cells*

Beginning of differentiation of visual cells is first indicated at stage 22 when small cytoplasmic buds are seen projecting from the outer nuclear layer into the ventricular space between the sensory retina and the pigmented epithelium. All buds apically contain an achromatic vacuole which persists for only a short while as the buds elongate (figure 2). As this vacuole disappears another arises in the basal region of the majority of differentiating cells. Careful examination occasionally reveals the presence of both vacuoles in a few cells at the same time. By stage 24 beginning development of outer segments is clearly seen (figure 3). At this stage the developing visual cells do not stain deeply or differentially but two morphological types are easily distinguished. Most of these cells contain the second vacuole and others are without any vacuole at all. The former are prospective rods and the latter are prospective cones. At stage 26 morphological distinction between cones and rods is even more distinct. Single cones are much fewer than rods and consist of a thin myoid, a somewhat deeper staining ellipsoid, a small conical outer segment and do not have any vacuole at all. Rods are quite numerous, all of them have a vacuole in the myoid region of the inner segment, ellipsoid is not yet distinct and the outer segment is long but tapering. In the central part of the retina in some sections there are seen one or two developing double cones each consisting of two closely associated cells, one of which has a vacuole in the inner segment like the rods but its small outer segment is sharply pointed; the outer cell is cone-like. The former represents the accessory and the latter the principal members of the double cone. Occasionally present in some sections there is a developing accessory cone apparently without an associated principal cone. This suggests that the two members of a double cone probably differentiate separately and become associated with each other later. These features of visual cells of stage 26 tadpole can be seen in figure 4. Double cones are better formed by stage 28.

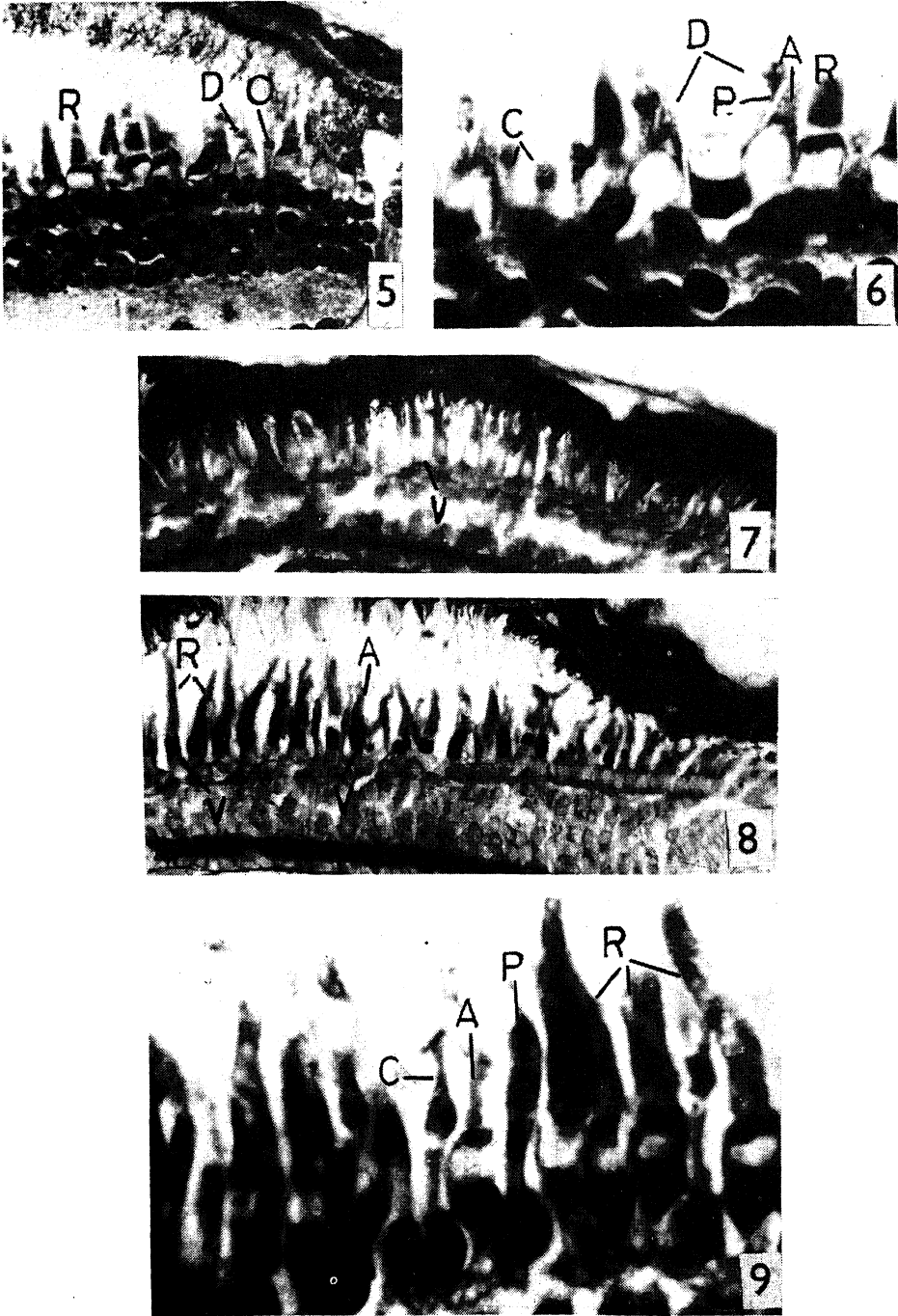
At stage 30 the three visual cell types including single cones, red rods and double cones are fairly well differentiated. Single cones are rather fragile cells with a small, conical outer segment and an inner segment consisting of a myoid and a peg-shaped ellipsoid. In an earlier publication (Niazi 1978), concerned with the ontogeny of photomechanical movements of pigmented epithelium, these cells were erroneously considered as green rods. Red rods are large with a long tapering outer segment and the inner segment consisting of a rectangular ellipsoid and a large vacuole in place of the myoid. Among the two members of double cones the principal cone resembles the single cone but has a longer myoid, and the accessory cone is a bulky cell having a rather long conical outer segment and a large barrel

shaped vacuole in the inner segment. The proximal side of the ellipsoid of accessory cone facing the vacuole is concave (figures 5, 6). With azan the outer segments of rods are now stained blue and those of single and double cones pink or orange. Red rods are still the most abundant, followed by single and double cones in this order.

The vacuoles in the inner segment of rods and accessory cones looked like paraboloid which is found in this region of some or all visual cells of many vertebrate species except mammals. Paraboloid does not take routine histological stains and appears like a vacuole. In all cases examined it has been found to contain a mass of glycogen which is visualized by specific stains for carbohydrates (Saxen 1955; Cohen 1972). Staining of retinal sections of stage 30 and older tadpoles, toadlets and adults with PAS revealed the presence of glycogen as dark bodies in the vacuoles of inner segment of both red rods and accessory cones confirming the paraboloid nature of these structures. Such dark bodies were not seen in the control sections predigested with saliva (figures 7, 8). Examination of retina from periphery towards the centre showed that glycogen accumulates gradually in the enlarging vacuole as the differentiating rods grow in size.

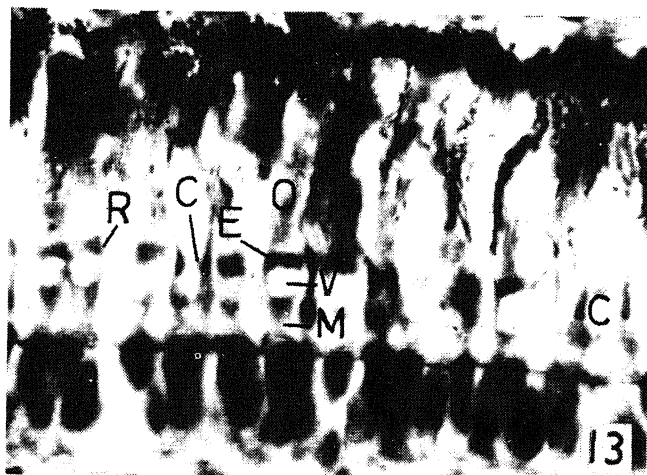
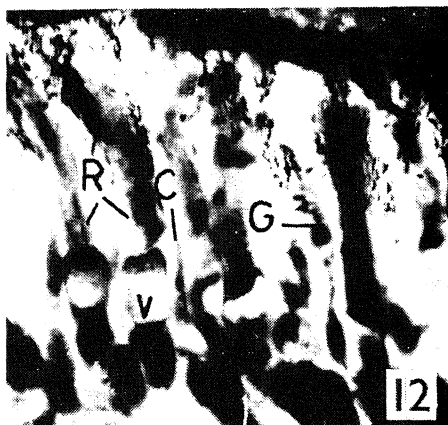
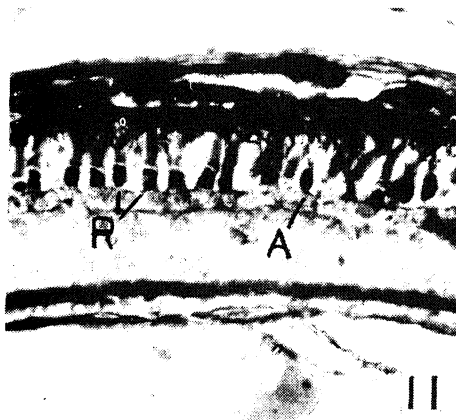
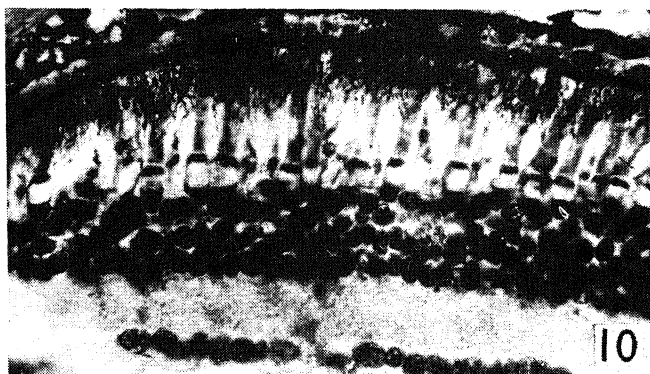
The period between stage 30 and 38 is one of rapid growth of the tadpole and increase in diameter of the eye ball as the retina stretches and expands. Visual cells grow in size as may be seen in figure 9 which shows these cells in a stage 34 tadpole. Fusion at the level of nuclei between an accessory and a principal cone to form a double cone is also indicated in this figure. It may be noted that rod outer segments are still tapering. They become cylindrical and reach up to the pigmented epithelium by stage 36 (figure 10). The number of visual cells increases greatly but the most notable increase is in the number of single cones, so that by stage 36 they are about as many rods and the tadpole retina becomes truly duplex (figure 10). Double cones remain much fewer than cones and rods and are not found in the periphery of the retina. Paraboloid with its glycogen is still present in red rods and accessory cones (figure 11). This condition of visual cells persists unchanged up to stage 38.

Metamorphosis begins at stage 39 with the emergence of the left forelimb and ends at stage 43 when the toadlet crawls out of the water. The fourth type of visual cells called green rods appear during this period. A young newly formed green rod in the retina of stage 39 tadpole is shown in figure 12. It may be noted in this figure that red rods still contain the paraboloid and myoid is absent. A fully developed green rod has a long thin myoid, deeply staining ellipsoid and a cylindrical outer segment which is similar but shorter than that of red rods and reaches up to the pigmented epithelium. Ellipsoids of green rods are located at the level of about the middle of the red rod outer segments (see figure 17). Green rods remain fewer than double cones and like the latter are not found in the peripheral region of the retina. Several other changes also occur during metamorphosis. The outer limiting membrane becomes prominent and location of rod and cone nuclei with respect to this membrane becomes clear. The oval nuclei of red rods are positioned across the external limiting membrane and cone nuclei lie deeper. Cone outer segments become sharply pointed needle-like. An important change occurs in the structure of red rods in which myoid begins to develop as the paraboloid along with its glycogen shrinks (figures 13, 14) and ultimately disappears (figures 15, 16). However, the paraboloid of the accessory member of double cones is not affected by metamorphosis (figures 14, 15). Red rods of adult toad consist of a cylindrical

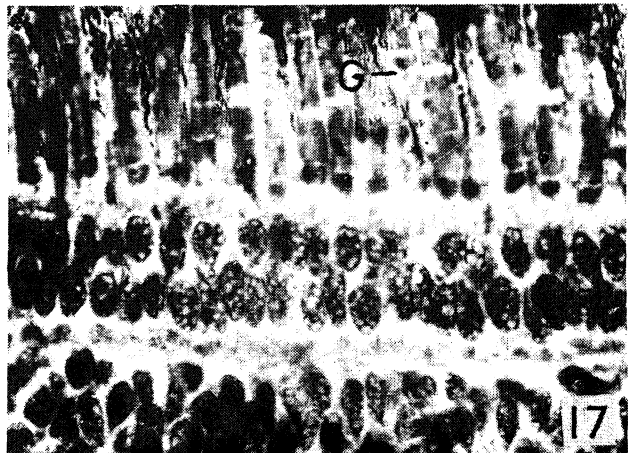
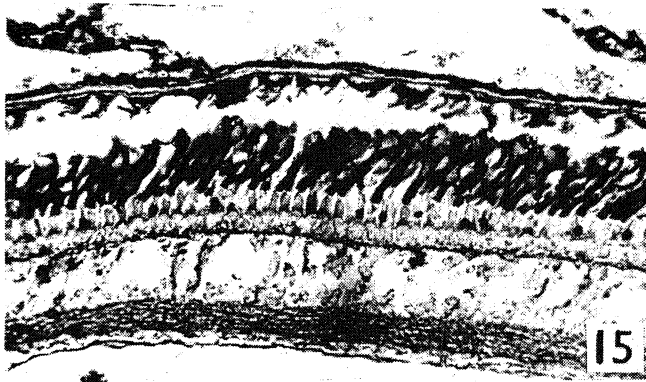
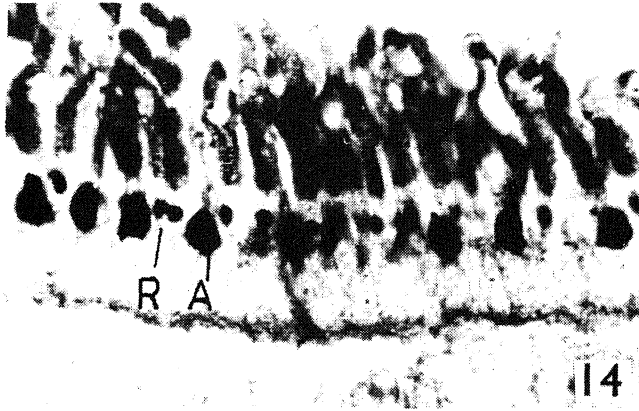


Figures 5-9. For caption see page no. 429.

myoid, deeply staining ellipsoid and a long uniformly cylindrical outer segment whose apex is surrounded by the cellular processes of the pigmented epithelium. During the latter part of and after metamorphosis the number of visual cells



Figures 10–13. 10. Retina of a stage 36 tadpole ($\times 400$). 11. Section of retina of a stage 36 tadpole stained with PAS; glycogen appears as dark bodies in paraboloids of red rods and accessory cones ($\times 200$). 12. Visual cells of a stage 39 tadpole ($\times 1000$). 13. Visual cells of a metamorphosing stage 42 tadpole. Note myoids developing between the paraboloid and nucleus of red rods ($\times 1000$). (A, Accessory cone; C, single cone; E, ellipsoid; G, green rod; M, myoid; O, outer segment; R, red rod; V, paraboloid).



Figures 14–17. 14. Section of retina of a toadlet (stage 43) stained with PAS. Glycogen appears as dark bodies. Note its reduction in the paraboids of red rods (R) but not in those of accessory cones (A) ($\times 1000$). 15. Section of retina of an adult toad stained with PAS. Note presence of glycogen in the paraboids of accessory cones. It has disappeared in red rods ($\times 400$). 16. Red rods of an adult toad stained with PAS. Note presence of myoid (M) in place of paraboid ($\times 400$). 17. Retina of adult toad. Note predominance of massive red rods ($\times 400$). (G, Green rod).

increases greatly but this time the most remarkable increase is in the number of red rods transforming the retina into a predominantly rod retina (figure 17).

4. Discussion

The observations suggest that the entire process of développement of visual cells in *B. melanostictus* from after hatching up to adult stage is divisible into 4 overlapping phases. During the first phase, which begins soon after hatching, single cones, red rods and double cones differentiate into morphologically distinct types but the red rods remain the most numerous. During the second phase, rapid increase in the number of cones makes the retina truly duplex, rod outer segments attain cylindrical shape and the number of double cones also increases. The first and second phases more or less coincide with the premetamorphic and prometamorphic periods, respectively, of the anuran larval life as defined by Etkin (1968); the former ends with the growth of hindlimb buds and the latter includes the growth phase of the tadpoles up to the onset of metamorphosis. The third phase includes the period of metamorphic climax during which green rods appear and larval red rods are transformed into adult type. Photomechanical movements of the pigment of the retinal pigmented epithelium in response to light and darkness also attain the maximum extent during this phase (Niazi 1978). Tremendous growth in the number and size of red rods, occurring mainly after metamorphosis, marks the final phase.

A most significant feature revealed by the study concerns the process of development of red rods in this species. As these cells differentiate in the tadpoles, instead of the myoid a paraboloid, containing a glycogen mass, develops in their inner segment and persists throughout larval life. During metamorphosis it shrinks and finally disappears as the growing myoid takes its place transforming the larval red rods into adult type. To our knowledge, the occurrence of a paraboloid in the red rods at any stage of development in any anuran species has not been reported before. Also, we have not come across any report about any species in which the paraboloid present in any visual cell in the juvenile or larval stage disappears with age or during metamorphosis. However, its occurrence in the larval red rods may not be a feature specific to *B. melanostictus* alone. A paraboloid-like structure in the red rods of *B. regularis* tadpoles is evident in figures 9 and 11 of El-Mekkawy *et al* (1984-85) although the authors have neither labelled it in the figures nor mentioned it in the text.

The paraboloid is found in one or more visual cell types of many species from fishes to birds but its function remains undetermined. A lens-like function has been suggested by different workers (Walls 1942; Saxen 1955; Cohen 1972). Saxen (1955) has convincingly argued against its glycogen being a source of energy for photomechanical movements of the visual cells in response to changes in illumination. Recently, Niazi (1981) did not find any apparent difference between the paraboloids of red rods or accessory cones of dark and light adapted retinas of tadpoles and adults of *B. melanostictus*. Whatever be the function of this organelle, it may be noted that in this toad while the paraboloid of rods disappears during metamorphosis that of the accessory cones remains unchanged. This suggests that whereas the function of accessory cones may be the same throughout life that of red rods of the tadpoles and adults may not be identical.

In *Necturus* the paraboloid is present in all visual cells and oil droplet in none. According to Howard (1908), in this urodele the precursor vacuole of the oil droplet gives rise to paraboloid instead. *B. melanostictus* also lacks the oil droplet but its precursor vacuole does appear in all visual cells at the bud stage of differentiation. However, it soon disappears leaving no trace. With respect to the origin of the paraboloid in this toad our observations are similar to those of Saxen (1956), who had found that this organelle of accessory cones in *R. temporaria* and *Triturus vulgaris* develops from a second vacuole that arises later and more proximally than the progenitor of the oil droplet.

In ranid frogs and also *Xenopus*, cones are said to develop earlier and faster than rods (Cameron 1905; Saxen 1954; Muntz 1964); but the ultrastructural study by Nilsson (1964) showed that in *Rana pipiens* both types differentiate simultaneously. In *B. melanostictus* also, cones and rods begin to develop at the same time but majority of the initially differentiating cells are prospective rods as indicated by early appearance of the paraboloid precursor in them. Cones catch up with rods numerically sometime after stage 30 making the retina duplex for the rest of the larval life.

Evidence from studies on frogs (Saxen 1954; Nilsson 1964) and newts (Saxen 1956; Keefe 1973) favours the view that a double cone is formed by fusion of a cone progenitor with a rod progenitor. Our observations also support this view: cone origin of the principal cone is suggested by its morphological similarity with single cones, and presence of the paraboloid in both larval red rods and accessory cones supports the origin of the two from similar progenitor cells.

Saxen (1954) had observed that even in very young tadpoles of *Xenopus* visual cells are already almost like those of the adults, but in *R. temporaria* the adult condition is attained only at the end of metamorphosis. According to him, since both the tadpoles and adults of *Xenopus* are aquatic, inhabiting the same photic environment, the visual system developed in young tadpoles is good for the entire life. In contrast, *Rana* tadpoles are aquatic but adults are amphibious with aerial vision. Therefore, some further development and changes must occur in the visual system of the tadpoles to make it suitable for the adult. In addition to great increase in the number and size of cones and rods the changes that occur during metamorphosis include the development of green rods and reduction in the number of double cones. Green rods are said to be responsible for positive phototactic response of adult frogs to blue light (Donner and Reuter 1962; Muntz 1964). The difference between the habits and habitats of tadpoles and adults of *B. melanostictus* is even greater; the former are aquatic and diurnal but the latter are strictly terrestrial and mainly nocturnal. The adult condition of the visual system in this species also is attained only at the end of metamorphosis and it involves development of green rods, structural change in the red rods and tremendous increase in their number and size. Development of the visual system first gives rise to a duplex retina good for photopic vision of the tadpoles before rods take over to make the retina suitable for scotopic vision of the adult.

Acknowledgement

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Figures 5–9. 5 and 6. Visual cells of a stage 30 tadpole (5 × 400; 6 × 1000). 7 and 8. Sections of retina of a stage 30 tadpole stained with PAS. 7. Control section stained after digestion with saliva. 8. Section stained without predigestion. In 8 glycogen appears as dark bodies in paraboloids of accessory cones and red rods. In 7 paraboloids are unstained (× 1000). 9. Visual cells of a stage 34 tadpole (× 1000). (A, Accessory cone; C, single cone; D, double cone; P, principal cone; R, red rod; V, paraboloid).

Female accessory glands and sperm reception in Tingidae (Heteroptera)*

DAVID LIVINGSTONE and M H S YACOOB

Division of Entomology, Bharathiar University, Coimbatore 641 046, India

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Abstract. Studies on the anatomy of the female internal organs of reproduction and the intromittent organs of 34 species of Tingidae confirm the absence of spermathecae. The accessory glands are vesicular and primitively unpaired. There is no vermiform gland and the term pseudospermatheca, introduced by Carayon in heteropteran literature, is a misnomer. The lateral oviduct and all the 7 pedicels of each ovary develop a permanent swelling in the middle that receive the spermatozoa, syringed into them through the minute pores of the armature of ejaculatory pouches of the endotheca, when it is lodged inside the bursa during copulation. The ejaculatory pouch and the bursa are so designed and adjusted for the purpose of sperm transmission. In a few species of Tingidae a median diverticulum that arises from the endotheca plugs the unpaired vesicular accessory gland, preventing wrongful lodging of the ejaculatory pouch and such a mechanism, not known so far, has been described as a unique feature. A scheme to trace the evolution of accessory gland, from a median unpaired contacaderine condition, to a paired independent gland, having its opening either in front or behind the lateral oviduct, has been detailed.

Keywords. Tingidae; accessory glands; intromittent organ; sperm reception; origin; evolution.

1. Introduction

Typically, the female internal organs of reproduction of Tingidae consist of a pair of laterally placed ovaries, each consisting of 7 telotrophic ovarioles and each ovariole terminally extending further as a fine terminal filament. All the 7 such filaments from each ovary unite at the anterior margin of the mesothorax to form a suspensory ligament. Both suspensory ligaments continue further up to the head capsule and then unite to form a common suspensory ligament that enters the head capsule, running dorsal to the suspensory ligament of the principal salivary gland that gets fastened to the frontal ridge. Livingstone (1967a) had suggested that it may have direct connection with the endocrine glands. All the 34 species of Tingidae examined in the present investigation show consistency in the number of ovarioles, as reported by Carayon (1950), Woodward (1950), Sharga (1955) and Livingstone (1967b) in other species of Tingidae. However, Drake and Davis (1960) had reported only 5 ovarioles in *Cantacader* sp.

Not more than one egg, with fully formed chorion, has been found in each ovariole and not more than 5 ovarioles in each ovary remain in such a condition at a time. All the 7 pedicels are similar. They are elongate, slender and visibly dilated in the middle. It is a permanent feature. The calyx communicates with the pedicels in front and lateral oviduct behind. The lateral oviduct also has a permanent dilation in the middle. Both the lateral oviducts join to form the common oviduct

*Contribution No. 69.

of Drake and Davis (1960) and Eguagie (1976), here referred to as the bursa copulatrix. The length of the bursa varies considerably from species to species and it leads to the exterior through a very short vagina, otherwise known as the genital chamber. In Cantacaderinae however, Drake and Davis (1960) described a genital chamber into which the two lateral oviducts are reported to open independently and a dorsal sac lying transversely and opening into it at its anterior margin.

The bursa copulatrix gives rise to a pair of vesicular accessory glands which may open into it either independently or through a common duct. The site of opening of this accessory gland varies considerably and that necessitates a discussion on the evolutionary trend of its morphology and disposition. This gland has been described as spermatheca by Pendergrast (1957) and Eguagie (1976). In this context, a discussion on the structural features of the intromittent organ also has been considered imperative.

2. Materials and methods

Live adult females of the following 34 species belonging to 23 genera and 2 subfamilies of Tingidae were immobilized in molten wax for microsurgery in insect Ringer.

Tingidae: Species examined

(1) *Aconchus urbanus*; (2) *Agramma graminii*; (3) *Agramma hupehanum*; (4) *Ammianus ravanus*; (5) *Cantacader quinquecostatus*; (6) *Cochlochila bullita*; (7) *Corythauma ayyari*; (8) *Corythauma gibbosa*; (9) *Cysteochila incolana*; (10) *Cysteochila javansis*; (11) *Dasytingis rudis*; (12) *Dasytingis semota*; (13) *Dictyla karnatica*; (14) *Dulinius conchatus*; (15) *Eteoneus cinchonii*; (16) *Habrochila laeta*; (17) *Haedus grewii*; (18) *Lasiacantha justiciaii*; (19) *Lasiacantha peristrophii*; (20) *Lasiacantha ruellii*; (21) *Naochila nigra*; (22) *Naochila minuta*; (23) *Perissonemia ecmeles*; (24) *Physatocheila asiatica*; (25) *Phaenotropis cleopatra*; (26) *Pontanus puerilis*; (27) *Stephanitis charieis*; (28) *Stephanitis macranthaii*; (29) *Stephanitis typica*; (30) *Tingis buddleiae*; (31) *Tingis tomentosii*; (32) *Teleonemia scrupulosa*; (33) *Urentius euonymus*; (34) *Urentius hystricellus*.

The internal organs were extirpated and fixed in warm aqueous Bouin's fluid for 12 h. They were then washed thoroughly in several changes of 70% alcohol, stained in Ehrlich's haematoxylin-eosin, dehydrated, cleared in cellosolve (2-ethoxy ethanol) and mounted in DPX. Materials thus fixed were also prepared for microtome following Peterfe's celloidin-paraffin embedding procedure and sections of 6 μ m thickness were stained in Ehrlich's haematoxylin-eosin, dehydrated in isopropyl alcohol and mounted in DPX.

The chitinous structures of the intromittent organs of all the 34 species were studied by microsurgery in glycerine, after treating the genital capsules in 10% KOH in varying lengths of time, depending on the intensity of sclerotization and washing thoroughly in 5% acetic acid. Such dissected materials were washed in water, dehydrated and cleared in several changes of cellosolve and mounted carefully to prevent distortion, in polyvinyl-lactophenol.

In order to ensure the position of the endotheca of the intromittent organ inside the bursa of the female during copulation for studying the manner of sperm transmission, copulating pairs were plunged into hot alcoholic Bouin's fluid that

caused instantaneous death. Such fixed insects were dissected carefully and their reproductive systems in copula extirpated, washed in 70% alcohol, stained in Ehrlich's haematoxylin-eosin, cleared in cellosolve and mounted in polyvinyl lactophenol. Such mounts as well as the stained sections were photomicrographed.

3. Results

3.1 Ovariole

Not more than 5 eggs have been found ovulated into the pedicels of each ovary and not more than one egg is found in the lateral oviduct at any one time. The maximum number of ovulated eggs is found in *A. ravanus*, *D. rudis* and *P. ecmeles* (figure 1L). In the teneral adults, the germarium and vitellarium are not clearly delineated and only 3 days after emergence alone the basal egg nodule begins to differentiate, with the differentiation of the follicular epithelium. The penultimate egg differentiates only after the basal egg has completed formation of chorion and opercular apparatus.

3.2 Pedicel

The pedicel (figures 1J and 2L) whose length varies considerably from species to species, develops a discrete dilation in the middle even much before the basal egg nodule is differentiated. In the grass tingids such as *A. urbanus*, *A. graminii* and *A. hupehanum*, that produce very short operculate eggs, the pedicels are very short whereas in species such as *A. ravanus*, *D. rudis*, *D. semota*, *P. ecmeles* and *P. puerilis* that produce long operculate eggs, the pedicels are fairly elongate. In all other species they are moderately elongate. This suggests that the pedicel length can be directly correlated with the chorionic collar height.

3.3 Lateral oviduct

The lateral oviduct is highly muscular (outer circular and inner longitudinal fibres) and is divisible into proximal and distal limbs, intervened by a very conspicuous permanent dilation (figure 2L), as in the pedicels. This dilation does not correspond to the short curled spermathecal organ, occupying a corresponding position, as described by Drake and Davis (1960) in *Cantacader* sp. Eguagie (1976) in the univoltine European species, *Tingis ampliata*, described that the onset of germinal division was associated with an enlargement of the middle region of both lateral oviducts and such enlargements persisted throughout the period of active oogenesis alone and was therefore considered to be a temporary seasonal feature. Such enlargements, as indicated in Eguagie's descriptions and diagrams, were also reported to be non-existent during other seasons and therefore not a permanent feature. Pedicels were not recognized by Eguagie in *T. ampliata*. In the present investigation all species have been found to be multivoltine, each raising 10–12 generations a year and the dilation in each of the pedicels and lateral oviducts is a permanent feature.

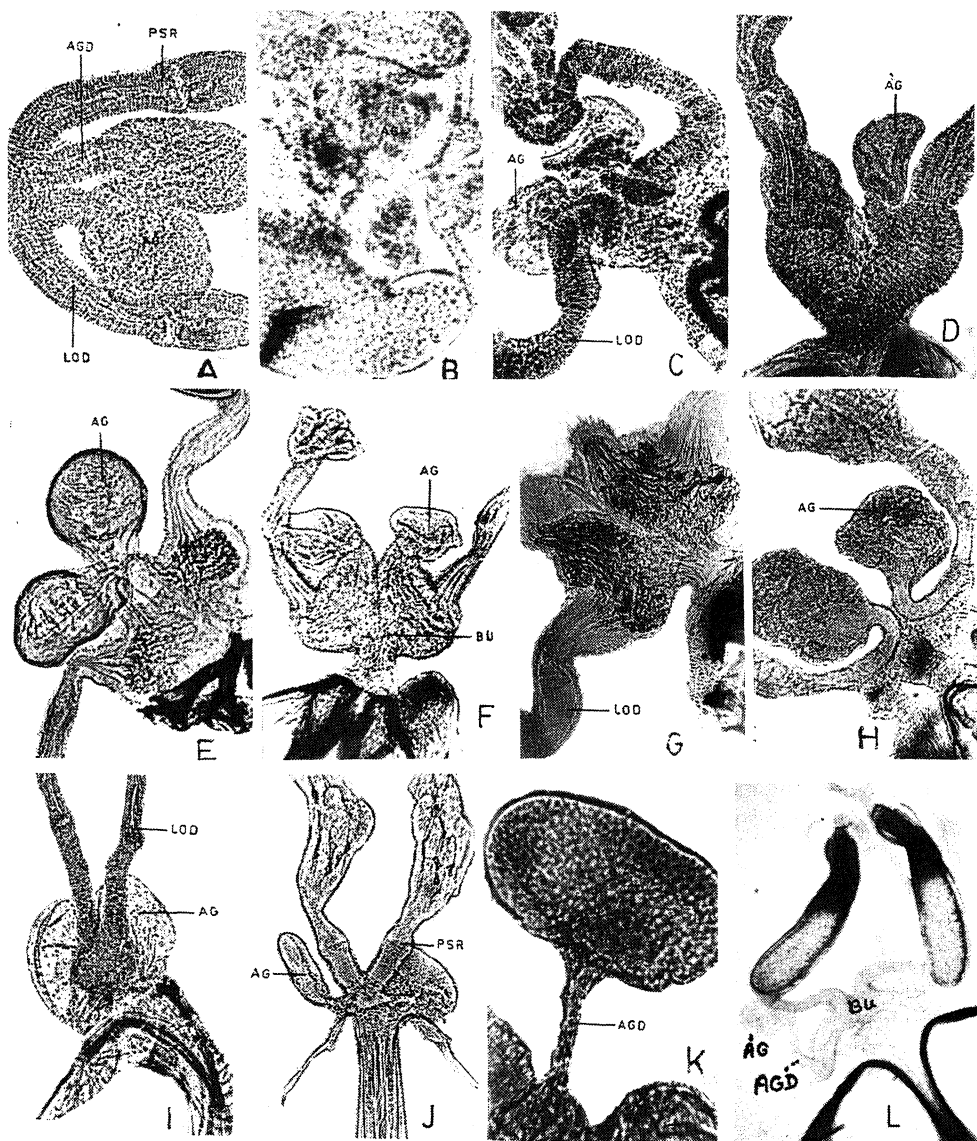


Figure 1. Female internal organs of reproduction. **A.** *C. incolana* ($\times 150$). **B.** *N. minuta* ($\times 150$). **C.** *D. karnatica* ($\times 150$). **D.** *C. ayyeri* ($\times 150$). **E.** *L. peristrophii* ($\times 150$). **F.** *L. ruellii* ($\times 150$). **G.** *L. justiciai* ($\times 150$). **H.** *U. hystricellus* ($\times 100$). **I.** *A. graminii* ($\times 150$). **J.** *E. cinchonii* ($\times 100$). **K.** *E. cinchonii* magnified view of an accessory gland ($\times 400$). **L.** *P. ecmeles* ($\times 75$).

Histomorphologically, the limb of the oviduct in front of the dilation has been found to be distinctly different from the limb behind it, in being free from intima and the pedicels have no trace of intima at all, as reported earlier by Livingstone (1967a). Sections of the ovaries of insects immediately after copulation reveal that the dilations of all the 7 pedicels and lateral oviducts remain highly distended and their lumen stuffed with spermatozoa (figure 3F,H), thus confirming the earlier

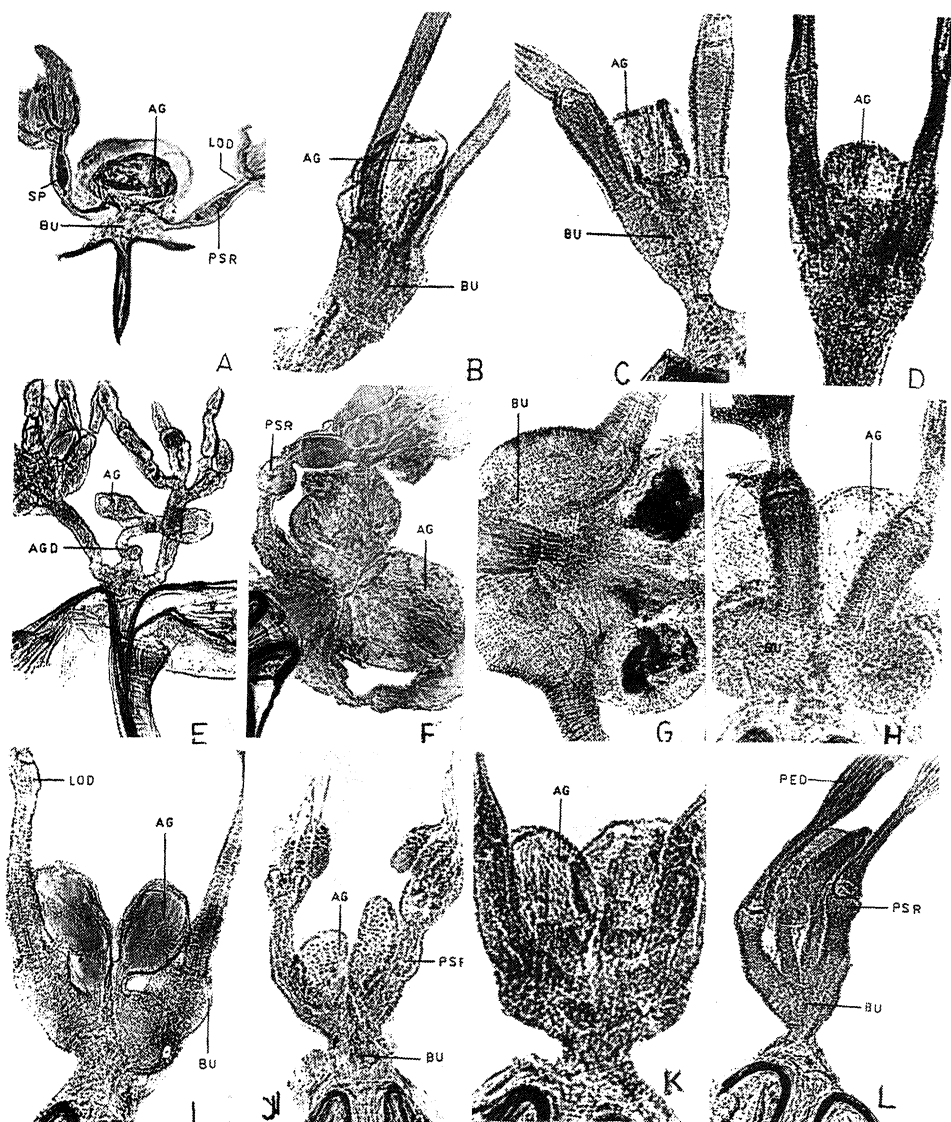


Figure 2. Female internal organs of reproduction showing accessory glands and oviducts showing spermatozoa in the principal and accessory receptacula semenis. A. *H. laeta* ($\times 50$). B. *A. ravanus* ($\times 100$). C. *D. semota* ($\times 100$). D. *P. puerilis* ($\times 150$). E. *D. conchatus* ($\times 100$). F. *C. bullita* ($\times 75$). G. *P. asiatica* ($\times 150$). H. *P. asiatica* showing crystallized secretion of accessory glands ($\times 150$). I. *T. scrupulosa* ($\times 100$). J. *H. grevii* ($\times 150$). K. *C. javensis* ($\times 100$). L. *C. gibbosa* ($\times 150$).

report of Livingstone (1967a) in *T. buddleiae*. For this functional reason, he designated the dilation of the lateral oviduct as principal receptaculum semenis and that of the pedicel as accessory receptaculum semenis. He further suggested that fertilization in Tingidae takes place much before chorion formation, as in cimicids and anthorcorids (Cobben 1968).

3.4 Bursa

Basally, the two lateral oviducts unite to form the common chamber into which the ejaculatory pouch of the intromittent organ (endotheca) is lodged during copulation (figure 3A, B, C, D). For this reason this common chamber was earlier designated as the bursa copulatrix by Pendergrast (1957) and the term common oviduct, thus far used to describe this region, therefore is a misnomer for Tingidae.

Examination of the position and extent of development of the bursa copulatrix in all species reveals a wide range of variation and such variation is found to be directly related to the nature and development of the endotheca of the intromittent organ and therefore they are both functionally species specific. In *A. urbanus* (figure 3A) and *H. laeta* (figure 4A) in which the ejaculatory pouch is not distinctly paired (figure 5), the bursa develops as a median spacious chamber (figure 2A). When the ejaculatory pouch is lodged inside this region during copulation, the sperms are syringed into the principal and accessory receptacula semenes of the lateral oviduct and pedicels respectively on either side. In all other species examined, the base of each of the lateral oviducts is appropriately enlarged to receive the ramus of ejaculatory pouch of the respective species and therefore serves as the functional bursa. Such an enlarged condition of the base of the lateral oviduct is more conspicuously developed in *P. asiatica* and *T. scrupulosa* (figure 2G, I). It is thus evident that the base of the lateral oviduct is capable of dilation according to the size of the ejaculatory pouch and for that reason this adjustment is species specific. This mechanism prevents the sperm from entering the accessory gland that is never found to contain sperms at any time, though it is closely associated with the lateral oviducts.

3.5 Accessory gland

In all the 34 species examined, it is found that occurrence of single or paired glands, either in association with the lateral oviduct or with the bursa, is a characteristic feature of Tingidae. They are always found to be vesicular and never occur as vermiform glands. Such paired vesicular glands, arising from the junction of the two lateral oviducts, have been described by Pendergrast (1957) and Eguagie (1976) as spermathecae in *T. ampliata* and the latter author, after having reported the presence of spermatozoa within this organ, homologised it with the receptaculum seminis of Carayon (1950), Southwood (1956) and Drake and Davis (1960). This vesicular gland also corresponds with the description given for a dorsal sac in *C. quinquecostatus* by Drake and Davis (1960) who homologised the same with a corresponding structure reported in Miridae.

Histological studies reveal that this vesicular gland has a secretory epithelium lined by intima and in a mature insect its lumen is filled with a homogeneous eosinophilic secretion which in a senescent insect tends to crystallize (figure 3G). In none of the 34 species examined, this vesicular accessory gland has been found to contain spermatozoa, though all these species have been observed to be polyandrous and multiple mating is a rule.

The position and manner of attachment of the accessory glands with either the bursa or the base of the lateral oviducts is not consistent, so also the morphology of accessory gland itself (figures 1, 2 and 6). In *C. quinquecostatus*, Drake and Davis

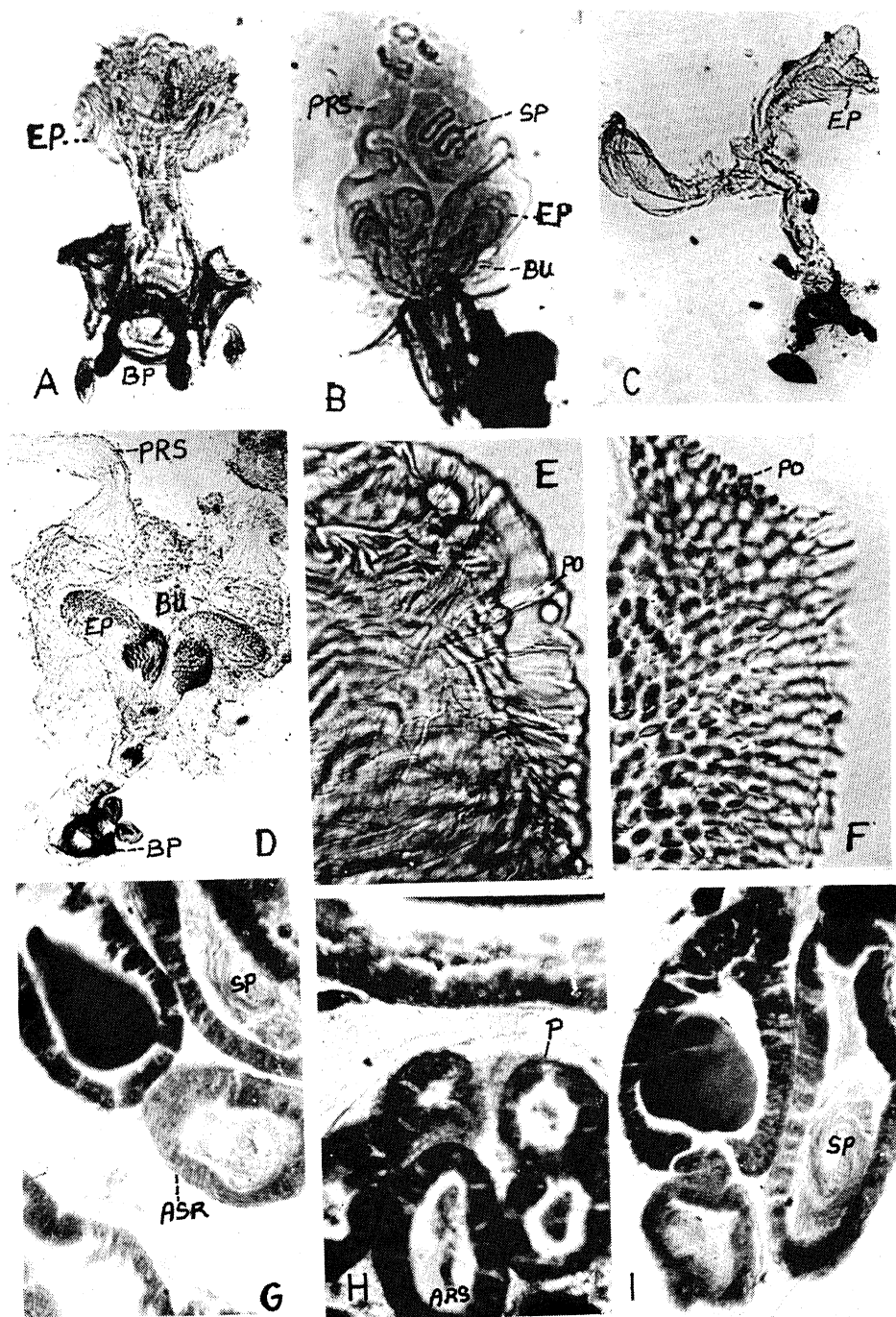


Figure 3. For caption, see p. 446.

(1960) had shown it as a median unpaired 'dorsal sac' and its functional role still remains obscure in this insect. In *H. laeta* too this gland occurs as an unpaired vesicle (figure 2A), but it is ventromedian in position, the two lateral oviducts

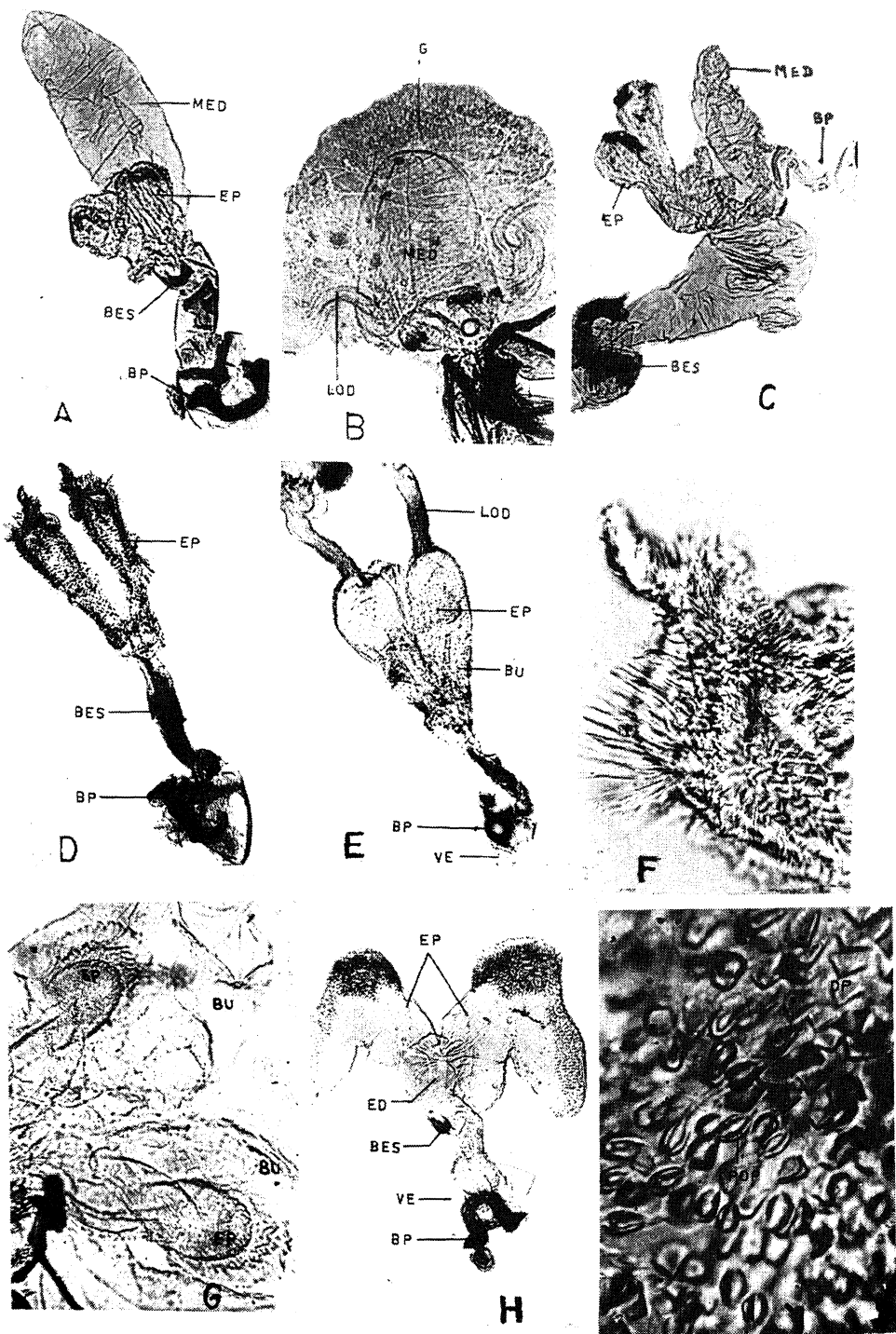


Figure 4. For caption, see p. 446.

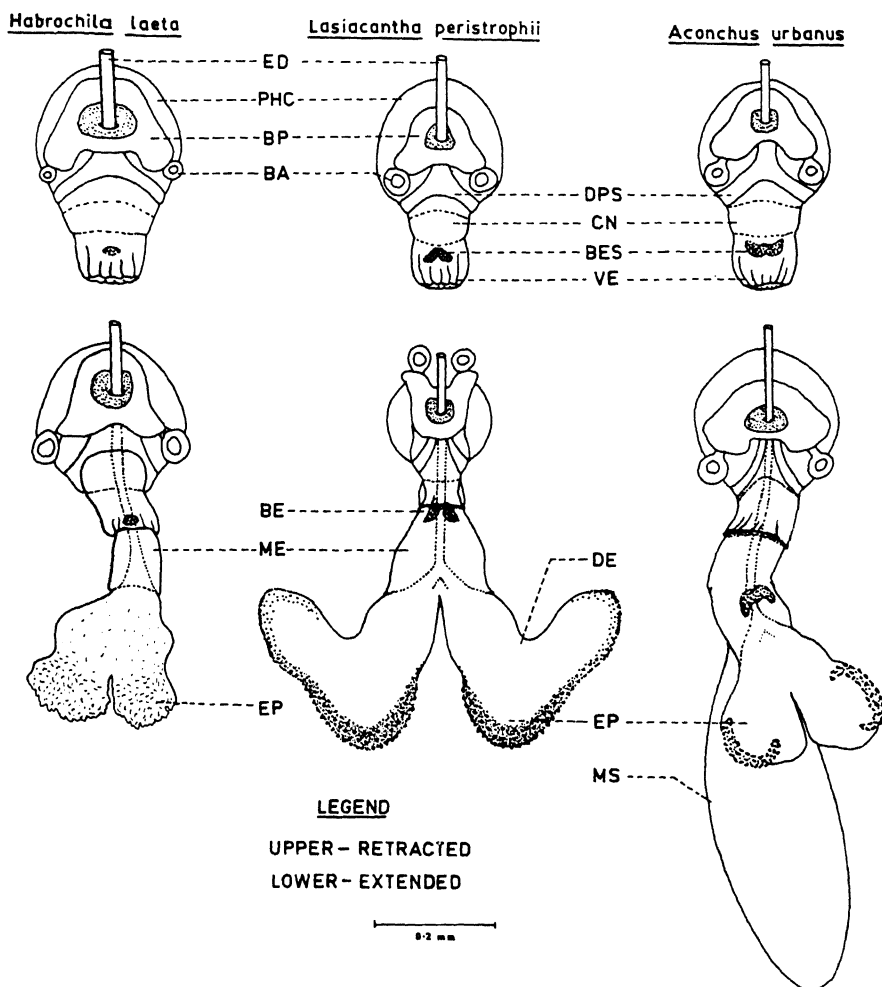


Figure 5. Intromittent organ — Modification types.

opening independently at its posterior corners (basal angles). Histology reveals that the lumen is filled with eosinophilic secretion but its posterior corners contain sperms enroute the principal receptaculum semenis of the lateral oviducts. During copulation, the unpaired but lobed ejaculatory pouch (figure 3) is lodged at the base of the vesicle and the sperms that are syringed directly into the lateral oviduct accumulate in the principal and accessory sperm repositories.

In *A. urbanus*, this median unpaired accessory gland vesicle is connected to the brief bursa by a short duct and the bases of the lateral oviducts remain enlarged. During copulation (figure 4A, B), while each of the two lobes of the ejaculatory pouch is lodged in the enlarged base of each oviduct, the median anterior sac of the middle segment of the endotheca (to be described below) remains lodged inside the unpaired accessory gland vesicle. This is a unique feature never met with in Heteroptera.

In *C. bullita*, the unpaired accessory gland apically develops a median longitudinal constriction (figure 2F) indicating the first stage of bifurcation of the

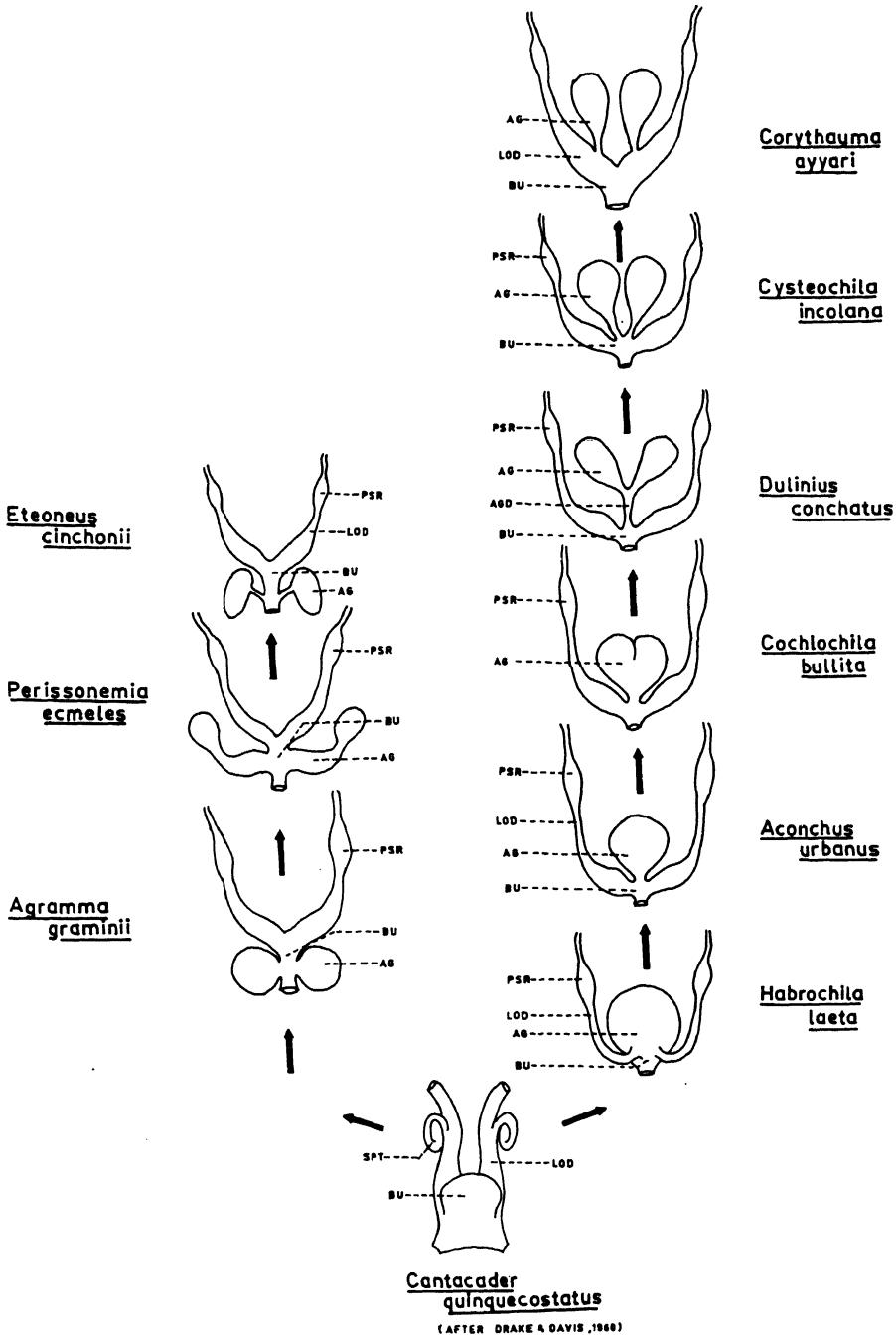


Figure 6. Tingid female accessory glands—Evolutionary trend.

(Abbreviations: AG, Accessory gland; AGD, accessory gland duct; BA, basal plate apodeme; BE, basiendotheca; BP, basal plate; BES, basiendothecal sclerite; BU, bursa; CN, conjunctiva; DE, distiendotheca; ED, ductus ejaculatorius; FG, female genitalia; EP, ejaculatory pouch; DPS, dorsal phallic sclerite; LOD, lateral oviduct; ME, midendotheca; MED and MS, median endothelial diverticulum; OP, pores of the ejaculatory pouch; PHC, phallic organ; P (PED), pedicel; PSR, principal receptaculum seminis; VE, vesica).

gland. In *D. conchatus*, the bifurcation is complete and the gland remains bilobed, connected to the bursa by a common duct (figure 2E).

Further evolution of the accessory gland from the *D. conchatus* type is indicated by the total separation of the two lobes as paired condition and establishment of independent opening for each lobe into the bursa, as seen in *C. incolana* (figure 1A). A still further evolution from this type leads to the shifting of each glandular vesicle towards the expanded base of the lateral oviduct as seen in *C. ayyari* (figure 1D) *S. cherieis*; *S. macranthaii*; *S. typica*; *T. scrupulosa* (figure 2I); *U. euonymus* and *U. hystricellus* (figure 1H). In these species each lobe of the ejaculatory pouch is lodged inside the expanded base of the lateral oviduct that serves as the functional bursa (figure 3B, C).

Yet another line of evolution met with in the accessory gland of Tingidae from the cantacaderine stock is the shifting of its position from the junction of the lateral oviducts posteriorward (figure 6). In *A. graminii* (figure 1I) the two vesicular glands are attached to the bursa without a conspicuous duct. Intermediate condition between this and the cantacader type is not known. In *p. ecmeles* (figure 1L) and *E. cinchonii* (figure 1J, H) the two accessory gland vesicles get much elongated and develop a long duct leading from a bulbous head.

On the basis of the morphology and disposition of the accessory glands in Tingidae, the following 10 categories could be recognised. However, unpaired tubular vermiform accessory gland, as described by Eguagie (1976) in *T. ampliata* has not been found in any of the 34 species of multivoltine Tingidae of southern India so far examined.

3.6 Scheme of evolution of female accessory gland in Tingidae

Type	Example
Dorsomedian vesicle	<i>C. quinquicostatus</i> (Drake and Davis 1960) (figure 6)
Ventromedian vesicle	<i>H. laeta</i> (figure 2A)
Anteromedian vesicle with a short duct opening into the bursa	<i>A. urbanus</i> (figures 1B and 6) <i>A. ravanus</i> (figure 2B) <i>D. rudis</i> <i>D. semota</i> (figure 2C) and <i>P. puerilis</i> (figure 2D)
Anteromedian vesicle apically bifid, with a common duct	<i>C. bullita</i> (figure 2F)
Anteromedian paired vesicles with common duct	<i>D. conchatus</i> (figure 2E) <i>D. karnatica</i> (figure 1C) and <i>P. asiatica</i> (figure 2; GH).
Anteromedian paired vesicles opening independently into bursa	<i>C. gibbosa</i> (figure 2L) <i>C. incolana</i> (figure 1A) <i>C. jawansis</i> (figure 2K) <i>H. grewii</i> (figure 2J) <i>L. justicialii</i> (figure 1G) <i>L. ruellii</i> (figure 1F) <i>L. peristrophii</i> (figure 1E) <i>N. minuta</i> (figure 1B) <i>N. nigra</i> <i>P. cleopatra</i> <i>T. buddleiae</i> (Livingstone 1967a)

Anteromedian paired vesicles opening independently into the expanded base of the lateral oviduct	<i>C. ayyari</i> (figure 1D) <i>S. charieis</i> <i>S. macranthaii</i> , <i>S. typica</i> <i>T. scrupulosa</i> (figure 2I) <i>U. euonymus</i> and <i>U. hystericellus</i> (figure 1H)
Paired vesicles opening behind the lateral oviducts	<i>A. graminii</i> (figure 1I)
Paired elongated vesicles opening behind the lateral oviducts	<i>P. ecmeles</i> (figure 1L)
Paired vesicles, each with long duct opening behind the lateral oviducts	<i>E. cinchonii</i> (figure 1J, K)

3.7 Intrômittent organ

The functional components of the intromittent organ (figures 4 and 5) of all the 34 species examined include the phallosoma (Phallosoma of Matsuda 1976) and the endosoma, the latter is greatly modified and remains withdrawn into the former at repose. The endosoma or the endotheca is divisible into the basal, the middle and the distal regions, corresponding to the basiconjunctiva, disticonjunctiva and vesica respectively of Matsuda (1976). The distal region is the ejaculatory pouch proper which is the continuation of the ductus ejaculatorius and could be considered as the expanded terminus of the ductus itself. This pouch is variously modified and could be correlated with the development of the bursa copulatrix as well as the expanded base of the lateral oviduct. The pouch carries minute pore bearing armature (figures 3E, G and 4I) for syringing out the sperms with force when it is lodged either inside the bursa proper or inside the swollen base of the lateral oviduct (figure 4B, E, G). After ejaculation it is withdrawn and kept within the Phallosoma. This pouch has been described earlier as the endophallic diverticulum by Drake and Davis (1960) and as Phallic spines by Livingstone (1967b). An ejaculatory bulb as a development of the ejaculatory duct, immediately in front of the basal plate foramen has also been described by Drake and Davis (1960) in *Corythuca pallipes* (Tinginae) and in *Anamastocoris coleopteratus* (Vianaidinae).

In *H. laeta* (figure 3A) and *A. graminii*, the ejaculatory pouch is distinctly bilobed but not prominently developed in the form of two appendages as found in *L. peristrophii* (figures 4H and 3D); *P. asiatica*; *C. bullita* (figure 3B, C); *T. scrupulosa* (figure 4D, E); *C. javansis* and most other species of Tinginae.

In *A. urbanus*, the middle region (disti-conjunctiva of Matsuda 1976) of the endotheca that occupies the region immediately behind the ejaculatory pouch, develops a finger shaped diverticulum (figures 4A and 5). During copulation, this diverticulum has been found to be lodged inside the unpaired median accessory gland vesicle (figure 4B) while the two lobes of the ejaculatory pouch are lodged inside the bursa, each lobe being directed towards the dilation (principal receptaculum semenis) of the lateral oviduct. This is considered as a special plugging device to prevent the entry of the sperms inside the accessory gland vesicle. However, the biology of this median endophallic diverticulum, as a unique feature in Heteroptera, awaits further exploration.

The armature of the ejaculatory pouch varies from species to species. It is either denticulate as in *L. peristrophii* (figure 4H, J) or filamentous, as in *T. scrupulosa* (figure 4D, F) or of mixed type (figure 3E, G). The armature further facilitates

securing firm grip on the wall of the bursa when the pouch is lodged in it during copulation. It also provides the required strength to stand the strain and stress in sperm ejection through the minute pores.

4. Discussion

The accessory glands and spermathecae in Heteroptera have never ceased to kindle the interest of Heteropterists ever since Carayon (1954) introduced the term 'Pseudospermatheca' in heteropteran literature and subsequently coined by Scudder (1959), Davis (1966, 1969), Wygodzinsky (1966), Cobben (1968), Louis and Kumar (1973), Cobben and Wygodzinsky (1975) and Matsuda (1976) to designate a pair of diverticula that arise from the base of the common oviduct in diverse groups of Heteroptera. Such a pseudospermatheca is also reported to be absent in several species of Reduviidae (Davis 1969).

Snodgrass (1935) defined a typical spermatheca as a simple sac like structure with muscular wall and slender duct and several other structures governed by this definition were generally considered as spermathecae (Ludwig 1926; Malouf 1933; Larsen 1938). Davis (1955) in mirids designated a similar structure that opens medially into the genital chamber as the spermatheca or 'seminal repository' and he further homologised this structure with a corresponding structure described by Ekblom (1926) and Kullenberg (1947) in Nabidae that never received sperms. Davis (1957) further described a long convoluted gland and homologised it with the spermatheca of other insects, but explained that it has modified to function as colleterial gland, instead of receiving sperms. A similar view was also maintained by Dupuis (1955). Subsequently, Scudder (1959), in Reduviidae, described a median 'spermathecal gland' and had suggested that this has undergone great variations in Heteroptera.

The understanding of the structure and function of the female accessory glands in Heteroptera became further complicated by the discovery of several tubular elements in Emesinae by Pendergrast (1957), Wygodzinsky (1966) and Cobben and Wygodzinsky (1975). Here, a median elongate, narrow tube was recognized as the true spermatheca. Scudder (1959) described a pair of bulbous structures, each with a short duct and he recognized them as spermathecae. The same paired structures were described by Wygodzinsky (1966) as subglobular, apically ampullate structures containing sperms and he considered them as glandular 'Pseudospermathecae'. Yet another vermiform, apically bifid structure found on the ventral surface of the common oviduct was also described by Wygodzinsky (1966) as pseudospermatheca, functioning as additional sperm repository. Apart from these, a pair of lateral finger shaped processes, arising from the vagina and containing an amorphous substance also have been reported by him. Such tubular glands in *Triatoma* and *Rhodnius* however, were recognized by Galliard (1935) and Pendergrast (1957) as true accessory glands.

In Cimicidae and Anthocoridae, a spermatheca is absent and the sperms are reported to be injected into the organ of Berlese or Ribaga's organ, situated inside and from this the sperms are known to migrate haemocoelically into paired seminal receptacles, otherwise described as Pseudospermathecae, located at the base of the lateral oviduct (Carayon 1952a, 1953a, b; Carayon and Villiers 1968; Davis 1956). Further, Carayon (1952b, c, 1954) had reported haemocoelic insemination in

Nabidae and in this group the sperms are known to accumulate in the pedicel. In these species a Pseudospermatheca is not known and a vermiform gland of unknown function was considered to be modified spermatheca (Carayon 1961). In *Triatoma* and *Rhodnius*, the expanded portion of the common oviduct representing the vagina of Snodgrass (1935) and bursa copulatrix of Pendergrast (1957) has been regarded as the temporary sperm repository by Khalifa (1950).

In Tingidae, a true spermatheca has been reported to be absent in the subfamily Vianaidinae and in this subfamily, the spermatozoa have been known to accumulate in the lateral oviducts (Drake and Davis 1960). Carayon (1954) further described in Tingidae a pair of diverticula as extensions of the lateral oviducts, functioning as sperm receiving organs and he termed them as 'saccus semenalis' or 'sperm sac' which correspond to the short curled 'spermathecal organ' of the lateral oviduct of *Cantacader* species, as described by Drake and Davis (1960). The descriptions given by Pendergrast (1957) and Eguagie (1976) for the paired vesicular structures found at the base of the lateral oviducts, naming them as spermathecae, in *Tingis ampliata* are exactly similar to the advanced type of accessory glands, as described here in the case of *C. ayyari*, all species of *Stephanitis* and several other species of Tingidae. However, the vermiform gland; as reported by Eguagie (1973) in *T. ampliata*, as the accessory gland is not found in any of the species examined in the present study.

The secretory nature of these vesicular structures, either paired or median, and the absence of sperms in any of them unequivocally establish the fact that a true spermatheca is non existent in Tingidae and confirm the functional role of these vesicular structures as accessory glands. The cantacaderine type of median dorsal unpaired accessory gland is considered here as the prototype that, in the course of evolution, has given rise to the paired condition of this gland and this has taken place in two directions—the *Corythauma* line on the one hand, in which the glands maintain a position anterior to the bursa and the *Eteoneus* line on the other, in which these glands have shifted their position posterior ward towards the bursa.

The ejaculatory pouch of the intromittent organ is differentiated with the concomitant functional organization of the bursa, in order to receive the corresponding ejaculatory pouch. The lateral oviducts and the pedicels, by their differentiation, are prepared to receive the sperms when injected into them from the ejaculatory pouch. The vesicular accessory glands do not participate in sperm transmission. These facts confirm the suggestion that direct insemination occurs without the aid of a spermatheca in Tingidae. Fertilization taking place before chorion formation by this insemination procedure in Tingidae, as in mirids, cimicids and anthocorids, is therefore a logical conclusion.

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Figure 3. Intromittent organs and sperm reception. **A.** *H. laeta*. Intromittent organ with the expanded ejaculatory pouches ($\times 100$). **B.** *C. bullita* in coupla showing the ejaculatory pouches, each lodged inside the expanded base ('Bursa') of the lateral oviduct and the sperms (SP) in the principal (PSR) and accessory receptacula semenes ($\times 75$). **C.** *C. bullita*. Intromittent organ with the ejaculatory pouches extended (refer figure 4G) ($\times 100$). **D.** *L. peristrophii* in coupla showing the ejaculatory pouches lodged inside the expanded base ('Bursa') of the lateral oviduct and directed towards the lateral oviduct ($\times 75$). **E.** *A. ravanus*. Magnified view of the ejaculatory pouch showing pores (PO) ($\times 400$). **F.** *C. quinquecostatus*. Magnified view of the ejaculatory pouch showing pores (PO) ($\times 400$). **G.** *H. laeta*. Longitudinal section through the ovariole after copulation showing the developing basal oocyte and the pedicel stuffed with spermatozoa (SP) ($\times 100$). **H.** *H. laeta*. Transverse section through the pedicels (P) showing sperms (SP) inside the accessory receptacula semenes ($\times 100$). **I.** *H. laeta*. LS of the ovariole through the pedicel showing spermatozoa ($\times 100$).

Figure 4. Intromittent organs of Tingidae. **A.** *A. urbanus*. Intromittent organ, with the median endophallic diverticulum and ejaculatory pouches extended ($\times 50$). **B.** *A. urbanus* in copula. The median endophallic diverticulum lodged inside the accessory gland vesicle and the ejaculatory pouches lodged inside the bases of the lateral oviducts ($\times 100$). **C.** *P. ecmeles*. Intromittent organ with the median endophallic diverticulum and ejaculatory pouches extended ($\times 50$). **D.** *T. scrupulosa*. Intromittent organ with the ejaculatory pouches extended ($\times 50$). **E.** *T. scrupulosa* in copula. The ejaculatory pouches each being lodged inside the expanded base of the lateral oviduct ('Bursa') ($\times 50$). **F.** *T. scrupulosa*. Magnified view of the ejaculatory pouch showing fine filamentous processes with pores for syringing out the sperms ($\times 400$). **G.** *C. bullita* in copula. The ejaculatory pouches, each lodged inside the expanded base of lateral oviduct (Bursa) ($\times 100$). **H.** *L. peristrophii*. The intromittent organ with the ejaculatory pouches extended and the ductus ejaculatorius connected to both of them ($\times 50$). **I.** *L. peristrophii*. Magnified view of the ejaculatory pouch showing denticulate processes bearing pores for syringing out the sperms (refer figure 3D) ($\times 400$).

Stridulation in the coconut rhinoceros beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

A MINI and V K K PRABHU

Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581, India

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Abstract. The coconut rhinoceros beetle *Oryctes rhinoceros* produces different kinds of stridulatory sound under different conditions. Intense stridulations are made quite frequently by the male during courtship and mating attempts. Males also produce characteristic stridulations during aggressive encounters with other beetles, and distress stridulations, when disturbed manually. Females also stridulate, though less frequently. Sexually immature females produce feeble repellence stridulations while courted by males. Gravid females, when confined with males, are found to mimic the courtship and mating behaviour of the males, meanwhile producing intense stridulations resembling male courtship and mating stridulation. This behaviour, presumably has an ovipository motive and, to our knowledge, is the first instance of 'pseudomale' activity to be reported in insects.

Stridulatory mechanism comprises rubbing of a specialised region along the margin of the apex of the elytron—the pars stridens, against a series of striations—the plectrum, occupying the dorsum of the 7th abdominal tergite. Stridulation is possible with a single pars stridens, either of the left or right elytron, both being identical. No sexually dimorphic difference is apparent in the pars stridens. Plectral structure exhibits sexual dimorphism, being much prominent in the male.

Wing-locking is necessary to keep the elytra in the stridulatory position. Locking is effected by a longitudinal flange along the median side of one elytron (either the left or right, irrespective of the sex) fitting into a corresponding depression along the other. This differs from the reported cases in other coleopterans in which the flange of the left elytron extends under the right when locked.

Keywords. *Oryctes rhinoceros*; stridulation; pars stridens; plectrum; courtship.

1. Introduction

During our laboratory studies on the reproductive behaviour of the coconut rhinoceros beetle *Oryctes rhinoceros* it was observed that the sexually activated male beetles/attempting to mate with conspecific females produced loud stridulatory sound quite audible at a distance of about 150 cm. The beetle was found to protrude and retract his apical abdominal segments repeatedly in a rhythmic manner; the tergite of the segment just anterior to the pygidium was rubbed against the inner surface of the elytral tip backward and forward. Instances of elytro-abdominal type of stridulation have also been reported in the white pine weevil *Pissodes strobi* (Harman and Harman 1972), the curculio *Conotrachelus* (Gibson 1967), bark beetles of the genus *Dendroctonus* (Michael and Rudinsky 1972) and in certain scarabaeids (Richards and Davies 1977). In the scarabaeid *Heliocopris bucephalus* sound is produced by another mechanism in which hind coxa is rubbed against the floor of the coxal cavity (Narendran and Joseph 1978). The present paper describes the structures involved in the production of stridulatory sound in the scarabaeid *O. rhinoceros* and also distinguishes different kinds of stridulations as produced by these beetles under different circumstances.

2. Materials and methods

The beetles used for experiments were raised from field-collected third instar larvae sexed as per the method of Mini and Prabhu (1988) and reared individually on sterilized cowdung according to Mini and Prabhu (1985). Adults could be sexed easily by the presence of bare pygidium in the male as opposed to the bushy one in the female (O'Conner 1953; Nirula 1955). Also the pygidium is round in the male and conical in the female (figure 1A, B). From 30 days onwards of their emergence, the adults were fed ripe banana once a week. For this, they were kept singly in containers along with ripe banana slices for one-day and then returned to the medium.

To observe courtship and mating stridulations, each non-mated 40–50-day old male beetle was confined along with a 30–50-day old virgin female inside an open glass container (9 cm height \times 7 cm diameter) and watched closely for a maximum of an hour (Expt. I). This was replicated 10 times. Observations were also made with male beetles (40–50-day old) from which: (i) the 7th abdominal tergite was cut off fully; (ii) the elytral apex (that part of the elytra overlying the 7th tergite) was excised; (iii) apex of the right elytron alone was excised; (iv) apex of the left elytron alone was excised; (v) right elytron was excised completely; (vi) left elytron was excised completely; (vii) both hind-wings were excised completely. Five individuals were observed in each category.

To remove the 7th tergite, the beetles were held in hand, their elytral locking disengaged with the help of forceps inserted in between and keeping the elytra and hind wings upwards, the whole tergite was excised along its margin. The hind-wings and whole elytron were also removed in a similar manner. After the operations the remaining wings were brought back to their original position and the wing-locking was reestablished manually (where both elytra remained). The beetles were used for observations 15 min after operation.

To find out the relation between abdominal movements and sound production, dead beetles, 5 males and 5 females (within 24 h after death) were manipulated in such a way that similar movements were reproduced manually. For this purpose the beetles were held between the thumb and index finger and keeping the animal close to the ear the abdomen was pressed slowly and repeatedly to make its dorsum rub against the elytra (Expt. II). This was also repeated with dead beetles after removing their: (i) 7th abdominal tergite; (ii) elytral apex; (iii) right elytron; (iv) left elytron; (v) hind wings. Five males and five females were used in each category.

Female beetles (30–40-day old) were allowed to mate once with non-mated 40–50-day old males by confining them in pairs to the container for a maximum of 1 h daily or until mating took place, whichever was earlier. These mated females, upon reaching an age of 60–80 days were confined singly with 30–50-day old male and observed for 1 h to witness 'ovipository' stridulation (Expt. III). The females were then dissected out and the number of mature eggs counted.

Female beetles attained sexual maturity 23 ± 2 days after emergence (unpublished observation). Immature female beetles, 10–20 days after emergence, were confined each with a 40–50-day old non-mated male and watched for 1 h to observe repellence stridulation (Expt. IV).

To evoke male aggressive stridulation, 40–50-day old non-mated male beetles were confined in pair inside the container and observed continuously for 2 h (Expt. V).

Distress stridulation (Expt. VI) was induced in 40–50-day old non-mated male beetles (i) by holding lightly and touching roughly and repeatedly on their mouth parts with a camel hair brush and (ii) by pressing them hard between thumb and index finger for about 60 s and then releasing them upon table for watching. Experiments III–VI were each replicated 10 times.

Structural details on the dorsum of the 7th abdominal tergite and the inner surface of the elytral apex were studied by observing these parts excised from live beetles of both sexes, 10 males and 10 females, under a binocular dissection microscope. A thin coat of Quickfix (an adhesive cement manufactured by Wembley Laboratories, Delhi) was applied to the surfaces of study after gently scraping off the hairs with a needle, and when dried, film was peeled off to study the impressions cast on it, under the microscope.

The mechanism of wing-locking was studied by cutting across the elytra at its stridulatory zone in the locked condition and viewing the cut face under dissection microscope. Ten beetles each from both sexes were used for this purpose.

3. Results and discussion

Results of Expts. I–VI are given in table 1. Male beetles produced loud stridulatory sound during courtship and mating. While making this sound, he was found to exhibit rhythmic abdominal movements in which the penultimate tergite was scraped repeatedly against the elytral apex. Similar sound could be produced in dead beetles by manually reproducing identical movements. However, no sound could be produced in dead beetles and live beetles from which either the 7th tergite or elytral apex was extirpated. Thus it was evident that the sound producing devices were distributed partly on the dorsum of the 7th tergite and partly on the elytral apex and that sound was produced by rubbing one against the other.

3.1 Structure of the plectrum

The stridulatory structures associated with the abdominal tergite in different coleopterans have been referred to as plectrum (figure 1A, B) (Dumortier 1963; Gibson 1967; Harman and Harman 1972; Michael and Rudinsky 1972).

In the male *O. rhinoceros* dorsum of the 7th abdominal tergite revealed a striated appearance, being formed of numerous parallel rows of transverse ridges or striations. This area of striations commenced from a little behind the anterior margin and extended up to the posterior margin of the tergite. Laterally it extended about half the distance from the median line on either side and merged with a rather scaly area covered with hairs.

The ridges were highly prominent in the anterior region of the tergite where they were organised into two distinct zones—the zones of major striations (zmas), one on either side of the middle line. Lying medially, and laterally and posteriorly to these zones of major striations, was another zone—the zone of minor striations (zmis). The zones of major striations were formed of thicker, higher and well-separated ridges arranged in 5–12 rows with larger spacing in between. The size of these ridges decreased gradually so as to merge with the zone of minor striations. The zone of minor striations was formed of more closely packed rows of thinner, less higher and discontinuous ridges. There were very few hairs in the plectral region, especially along the mid-dorsal line.

Table 1. Stridulation in *O. rhinoceros* under different conditions.

Expt. No.	Beetle used/ treatment	Kind of stridulation observed	No. of beetles tested		No. of beetles stridulated	
			Male	Female	Male	Female
I	Normal mature male	courtship and mating stridulation	10		10	
	7th abdominal tergite removed from the male	—	5		—	
	Elytral apex removed from the male	—	5		—	
	Right elytron-apex alone removed from the male	—	5		5	
	Left elytron-apex alone removed from the male	—	5		5	
	Right elytron removed from the male	—	5		—	
	Left elytron removed from the male	—	5		—	
	Hind-wings removed from the male	—	5		5	
II	Dead beetles	Stridulation by manipulation	5	5	5	5
	7th abdominal tergite removed from dead beetle	—	5	5	—	—
	Elytral apex removed from dead beetle	—	5	5	—	—
	Right elytron removed from dead beetle	—	5	5	5	5
	Left elytron removed from the dead beetle	—	5	5	5	5
	Hind-wings removed from dead beetle	—	5	5	5	5
III	Gravid female	Ovipository stridulation		10		6
IV	Immature female	Repellence stridulation		10		7
V	Mature male	Aggressive stridulation	10 pair		3	
VI	Touching the mouth-parts of mature male with hair-brush	Distress stridulation	10		10	
	Pressing the mature male between the fingers	—	10		6	

Pleural ridges exhibited sexual dimorphism. In the female *O. rhinoceros*, the zones of major striations were less prominent being formed of smaller ridges less orderly arranged than in the male (figure 1B). In certain *Conotrachelus* species both sexes have identical plectrum (Gibson 1967) whereas in some *Dendroctonus* beetles, only the males have plectrum (Michael and Rudinsky 1972).

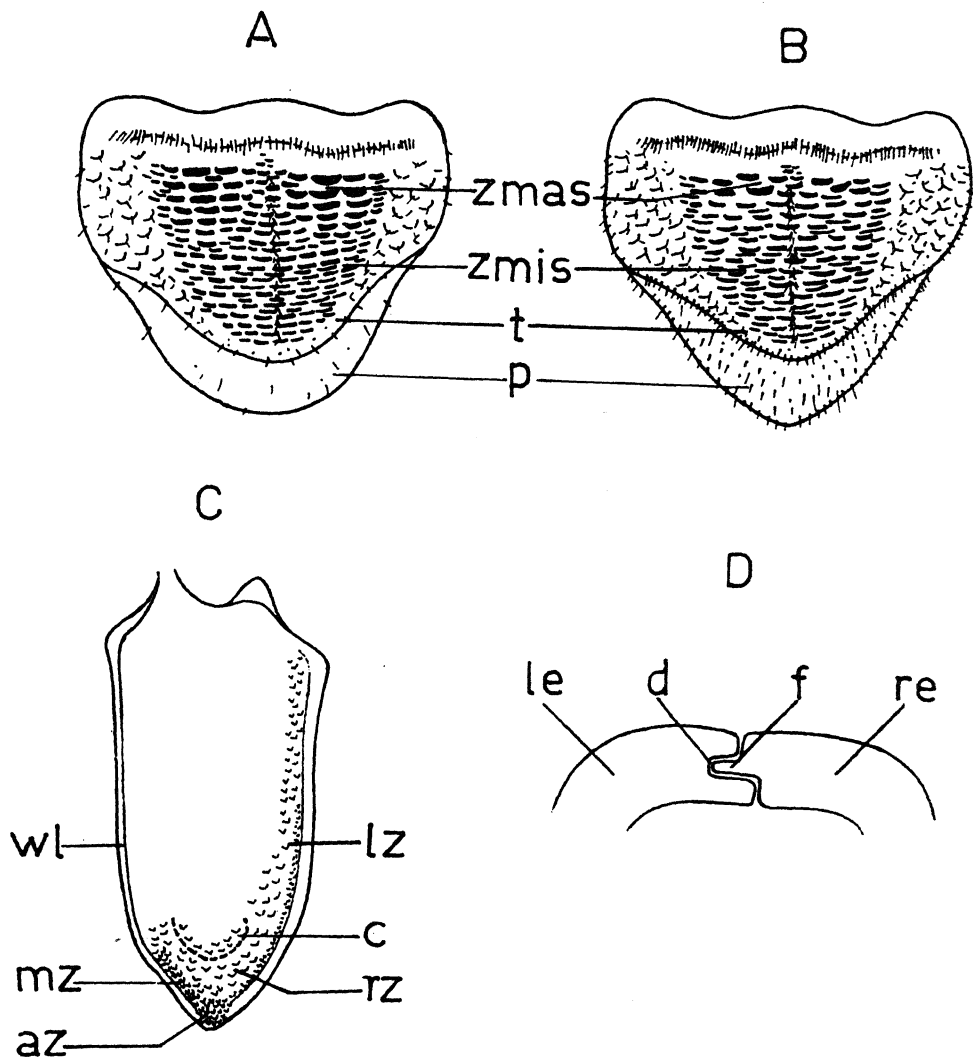


Figure 1. A. 7th abdominal tergite of male *O. rhinoceros* beetle showing the plectrum and pygidium. B. 7th abdominal tergite of the female showing the plectrum and pygidium. C. Left elytron-ventral view (after the removal of hairs). D. Cross-section of the elytra showing wing-locking mechanism. (az, Apical zone; c, concavity; d, depression; f, flange; le, left elytron; lz, lateral zone; mz, median zone; p, pygidium; re, right elytron; rz, rough zone; t, tergite; wl, wing-lock region; zmas, zone of major striations; zmis, zone of minor striations).

3.2 Elytral stridulatory structures

The inner surface of the elytral apex in *O. rhinoceros* was covered over with densely arranged posteriorly directed hairs. When these hairs were removed, a rough texture was unraveled; in the apical region of each elytron, there was a rough zone (rz) bearing numerous minute regularly arranged teeth-like projections, lying inner to the smooth rim of the elytron (figure 1C). In the median side of each elytron, this

zone reached only as far as the level of a concavity (c) found towards the elytral tip (mz). In the lateral side, this zone extended anteriorly through the whole length (1z). Towards the rim of the elytron, the teeth were much compactly aggregated and in the apical point of the elytron, this zone of closely packed teeth encroached up to the extreme edge, obliterating the rim (az).

Elytral structures associated with sound production have been described in several coleopterans as *pars stridens* (Dumortier 1963; Harman and Harman 1972; Michael and Rudinsky 1972) or *stridulitrum* (Gibson 1967).

When either the left or the right elytron was extirpated, live beetles could produce no stridulatory sound though stridulatory movements occurred normally. This was due to the absence of the wing-locking mechanism which normally kept the elytra firmly engaged with the plectrum during stridulatory movements. When one elytron was removed, the remaining elytron failed to remain in its normal position; it showed a tendency to rise up thus making it impossible to rub against the plectrum. If only the apex of the elytron was excised keeping wing-locking intact, the beetle could produce stridulatory sound with the help of the intact elytron. Sound could also be produced with a dead beetle having either the left or right elytron as this elytron was being brought into contact with the plectrum manually during manipulation. However, in certain male *Dendroctonus* beetles (Michael and Rudinsky 1972) and certain *Conotrachelus* weevils (Gibson 1967), the left elytron is essential for stridulation as the *pars stridens* is mostly confined to it. The weevil *Pissodes strobi* can however stridulate with a single elytron, either the left or right, since both are provided with closely similar *pars stridens* (Harman and Harman 1972), as in *O. rhinoceros*.

No sexually dimorphic difference could be observed in the elytral stridulatory areas.

3.3 Wing-locking

Wing-locking was effected by a longitudinal flange along the median side of one elytron (either the left or the right, which varied, irrespective of the sex) fitting into a corresponding depression along the margin of the other (figure 1 D). This condition differed from the reported cases in other coleopterans (Harman and Harman 1972; Gibson 1967) in which the flange of the left elytron extended under the right when locked.

The stridulatory areas did not extend into the wing-lock region. So there was no overlapping of stridulatory areas of the two elytra when they were interlocked; they occupied identical positions in relation to the plectrum and contributed equally towards stridulation.

3.4 Role of hind-wings in stridulation

Hind-wings were not essential for stridulation. Even after both hind-wings were removed, live beetles, and dead beetles upon manipulation, could produce stridulatory sound though the sound was distinctly different from that produced when hind-wings were present.

The left hind-wing was suspected to be functioning as a bridge or resonator in *Conotrachelus* (Gibson 1967). In *O. rhinoceros* the hind-wings were kept folded

beneath the elytra leaving a 'Λ' shaped gap between the folded apices through which the plectrum could communicate with the elytra; this space might have served as a resonating column.

3.5 Mechanism of sound production

During protraction of the last few abdominal segments (6th-8th), the plectral ridges scraped against the elytral apex producing a pulse of sound. During the subsequent retraction of the abdominal segments the plectrum again engaged the pars stridens, producing another pulse of sound. These two pulses produced during the forward and backward strokes together constituted a chirp. As the strokes were repeated varying number of times, a series of chirps (2-5) were produced at a single stretch constituting a chirp-series. The number of chirp-series produced by a courting male before achieving copulation varied from one to several.

The mechanism of sound production in *O. rhinoceros* is similar to that reported for certain cerambycids (Finn *et al* 1972).

3.6 Different kinds of stridulations

Stridulations that are of sexual significance have also been reported in a number of other coleopterans (Barr 1969; Claridge 1968; Mampe and Neunzig 1966; Michael and Rudinsky 1972; Wilkinson *et al* 1967). Apart from the courtship and mating stridulations, male *O. rhinoceros* were also found to produce stridulatory sound during aggressive encounters with other males. However, such aggressive stridulations could be observed very rarely, while observing male beetles confined in pairs inside the container. There were 3 instances during which the males met head-to-head and exerted pressure upon each other aggressively, with one or the other making abdominal movements associated with stridulatory sound. Such aggressive stridulations were different from sexual stridulations. Here the backward and forward strokes were separated by a detectable pause; also, the strokes were longer. These observations are in step with the audiospectrographic data from *Conotrachelus carinifer* (Gibson 1967) in which slower and more deliberate stridulations have the backward and forward strokes separated by a pause; in these, more stridulatory ridges per stroke are involved. In *Dendroctonus pseudotsugae* also, stress stridulation is different from sexual stridulation (Rudinsky and Michael 1972).

Live males could be induced to stridulate by touching forcefully on their mouth parts with a camel hair brush. Such distress stridulations were very feeble and short, resulting generally from a forward and a backward stroke coinciding with each stimulus and it could be detected only at close range. However if the beetle was pressed hard and then released, many made a series of chirps, closely resembling courtship stridulation. There was a time lapse of the order of a few seconds between the release of pressure and stridulation. Distress stridulations have also been reported in *Conotrachelus* (Gibson 1967) and *D. pseudotsugae* (Rudinsky and Michael 1972).

Thus, as reported for some other coleopterans (Finn *et al* 1972; Michael and Rudinsky 1972) *O. rhinoceros* was also capable of producing different kinds of stridulatory sound varying in intensity and duration, ranging from mild chirps

detectable only at a very close range to loud chirps detectable at a distance of about 150 cm.

3.7 Stridulation in female beetles

Female *O. rhinoceros* also stridulated, though less frequently. Young virgins (30–40-day old) did not stridulate while being courted by the male. However, mated females (60–80-day old) produced stridulatory sound comparable to male courtship and mating stridulation while they interacted with the males. These females chased the males, mounted them and exhibited rhythmic abdominal movements producing intense stridulatory sound, all in an exactly male-like manner. This behaviour, which could be regarded as 'pseudomale' behaviour, apparently enabled the female to circumvent the courting attempts of the male. However, there were several instances in which the females initiated this behaviour though there were no courtship threat from the males. One such female who was stridulating after mounting a male protruded the vagina and ejected out an egg along with the rhythmic abdominal movements. These observations together with the fact that this behaviour was exhibited only by gravid females suggested an 'ovipository' motive operating behind this behaviour. All these females when dissected revealed a number of mature eggs (9–18, with an average of 13.8 eggs). Stridulation by such gravid females may hence be regarded as 'ovipository', presumably having some link with oviposition.

Sexually unreceptive immature females (10–20-day old) when courted by the males, were sometimes found to make abdominal movements similar to that during stridulation. Feeble sounds could be heard at very close range; successive abdominal thrusts were widely separated. That is, instead of a series of backward and forward strokes occurring in rapid succession characteristic of 'ovipository' stridulation, there were isolated strokes during this repellence response, and these strokes were repeated at irregular intervals.

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Genetic and morphological variations in the Indian mackerel *Rastrelliger kanagurta* (Cuvier 1817) from the Goa region

MARIA R MENEZES, SANDEEP NAIK and MARIA MARTINS

National Institute of Oceanography, Dona Paula, Goa 403 004, India

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Abstract. The population structure of *Rastrelliger kanagurta* (Cuvier 1817) from two localities off Goa (Arabian sea) was assessed from genetic and morphological characters. Seventeen loci were examined from 8 enzymes, sarcoplasmic proteins and hemoglobins. The allele frequencies were not significantly different between the two localities. Nei's genetic distance value was 0.0017. The proportion of polymorphic loci at the 1% level was 47.1 and 52.9%. The level of genetic variation, estimated by average observed heterozygosity, was 5.8 and 6.6%. The sarcoplasmic protein-2 locus was found to be highly polymorphic. Significant ($P < 0.001$) departures from Hardy-Weinberg expectations were observed at this locus. About 67-89% of the variation in meristic characters was found to be within the groups.

Keywords. Genetic structure; heterozygosity; meristic characters; *Rastrelliger kanagurta*.

1. Introduction

The Indian mackerel, *Rastrelliger kanagurta* (Cuvier 1817) is a pelagic shoaling fish, widely distributed in the tropical Indo-west Pacific region. Mackerel landings in Goa (Lat. 14° 54' and 15° 48'N, Long. 73° 41' and 74° 20'E) constitute 20% of the mackerel catch in India (Doiphode 1974). The fishery is seasonal, lasting from September to March, and is restricted to a narrow coastal belt along some 400 miles along the west coast from Ratnagiri to Quilon in the south (Banerji 1962).

The results obtained so far from tagging experiments show that the movements of mackerel are of two categories, one showing limited migration and the other showing long distance, north-south migration (Venkataraman 1970). Rao (1962) indicated the possible existence of mackerel spawning grounds in several areas along the coast of peninsular India and the Andaman Islands.

The methodology of protein electrophoresis is now available to examine the genetic structure of fish populations which was previously impossible to study. With this method, a large number of individuals can be analyzed for many protein loci, and allele frequencies for these loci can be reliably estimated. Having accurate estimates of the frequencies of detected allelic variants is important since, as far as is known, genetic differences between populations at the intraspecific level are due in large part to frequency differences of alleles, rather than to alleles unique to different populations. This technique is used in the present study to estimate the genetic structure of *R. kanagurta* from two localities off Goa (Arabian sea). The total length was measured and the fin rays and gill rakers were counted for the specimens analysed.

2. Materials and methods

Specimens of *R. kanagurta* were collected from the fish landings at Panaji (north)

and Colva (south), in Goa during October–November 1988. The sampling localities are marked in figure 1. The fish samples were kept frozen at -20°C prior to analyses. Skeletal muscle, liver, heart and eye were dissected from individual specimens. The cell-lysate obtained by freezing and thawing was directly subjected to electrophoresis for phenotypic analysis. Starch gel electrophoresis was carried out by the procedure of Menezes and Taniguchi (1988). Detection of isozymes and nomenclature were as per the procedure of Allendorf and Utter (1979). The buffer used was citric acid aminopropylmorpholine (C-APM) of pH 6 (Clayton and Tretiak 1972) and Tris citric acid (T-C) of pH 8 (Shaw and Prasad 1970).

The list of enzymes, tissue specificity, buffer used and loci are given in table 1. A locus was considered polymorphic if the frequency of the most common allele was less than or equal to 0.99. The genetic basis of the protein variants were inferred from the observed banding patterns: (i) banding patterns were consistent with known molecular structures of analogous proteins of other fish and (ii) whenever the same locus was expressed in two or more tissues, the banding patterns of variants were consistent among tissues.

Average heterozygosity was determined by totalling the number of observed heterozygotes for each locus, dividing this by the total number of individuals in the sample, and averaging over all loci. Genetic divergence between populations was expressed by Nei's (1972) indices of genetic similarity (I) and genetic distance (D)

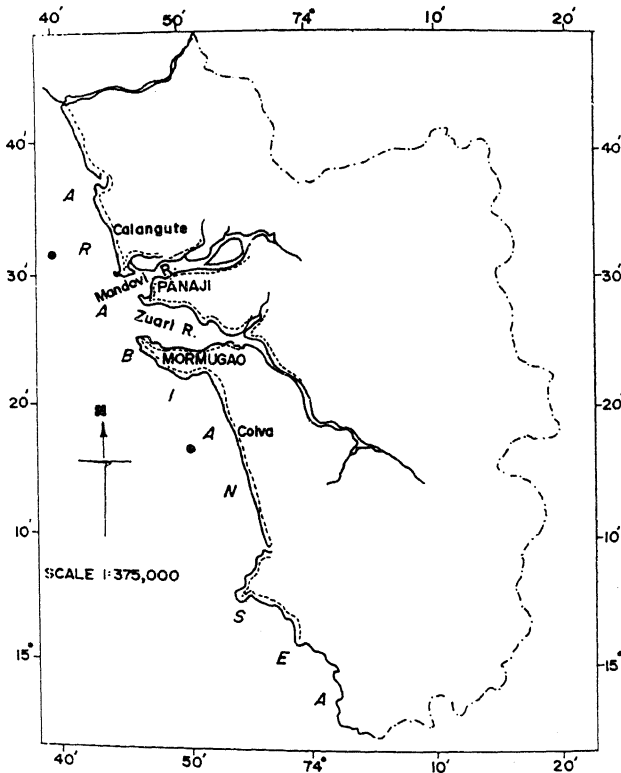


Figure 1. Map of Goa showing the sampling areas.

Table 1. List of enzymes examined, loci identified, buffer used and tissue assayed.

Enzyme	Enzyme number	Locus	Buffer	Organ
Glycerol-3-phosphate dehydrogenase (G3PDH)	1·1·1·8	G3pdh-B	C-APM	Liver
		G3pdh-A	C-APM	Skeletal muscle
Lactate dehydrogenase (LDH)	1·1·1·27	Ldh-C	TC	Eye
		Ldh-B	C-APM	Skeletal muscle, heart
		Ldh-A	C-APM	Skeletal muscle
Malate dehydrogenase (MDH)	1·1·1·37	Mdh-C	C-APM	Skeletal muscle
		Mdh-B	C-APM	Skeletal muscle
		Mdh-A	C-APM	Skeletal muscle
Isocitrate dehydrogenase (IDH)	1·1·1·42	Idh-B	C-APM	Liver
		Idh-A	C-APM	Skeletal muscle
Malic enzyme (ME)	1·1·1·40	Me	C-APM	Skeletal muscle
Phosphogluconate dehydrogenase (PGDH)	1·1·1·44	Pgdh	C-APM	Liver
Phosphoglucomutase (PGM)	5·4·2·2	Pgm	C-APM	Liver
Alcohol dehydrogenase (ADH)	1·1·1·1	Adh	C-APM	Liver
Sarcoplasmic protein (SP)		Sp-1	C-APM	Skeletal muscle
		Sp-2	C-APM	Skeletal muscle
Hemoglobin (HEM)		Hem	TC	Heart

C-APM, Citric acid aminopropylmorpholine pH 6; TC=Tris citric acid pH 8.

($D = -\log_e I$). Genetic distances reflect estimates of net codon differences between pairs of populations (Menezes 1990).

The *G*-test for independence (Sokal and Rohlf 1981) was used to compare allele frequencies among the two localities.

The total length of the fish specimens were measured (Laevastu 1965). The number of first dorsal (D1), second dorsal (D2), anal (A) and pectoral (P) fin rays, and gill rakers (GR) of the lower limb of the first gill arch were counted for the fish analysed from the two localities.

3. Results

R. kanagurta specimens from Panaji were in the size range of 16·5–22·5 cm with a mode at 20·5 cm, specimens from Colva ranged from 20·5–22·5 cm with a mode at 21·5 cm. Table 2 gives the range and means of the meristic counts of the specimens analysed. There was a highly significant ($P < 0·001$) added variance component (ANOVA, Sokal and Rohlf 1981) among the two groups for the first dorsal, second dorsal and pectoral fin rays (table 3). The proportion of variation among groups (coefficient of intraclass correlation) was 0·11, 0·31, 0·25, 0·55 and 0·33 for gill rakers, first dorsal, second dorsal, pectoral and anal fin rays respectively.

Eight enzymes, sarcoplasmic proteins and hemoglobins encoded by 17 loci (table 1, figure 2) were examined by electrophoretic method. Among 17 loci, 9 were polymorphic in Panaji sample while 8 in Colva sample. Diagrammatic representation of the relative electrophoretic mobilities of the polymorphic enzymes are given in figure 3. Allele frequencies and chi-square values for the differences between

Table 2. Counts of meristic characters of *R. kanagurta* from two localities.

	Locality	
	Panaji	Colva
N	100	40
Dorsal		
D1	7-10 (9.02 ± 0.65)	7-9 (8.42 ± 0.55)
D2	9-13 (11.46 ± 0.89)	11-13 (12.1 ± 0.44)
Fin rays		
Anal	7-13 (11.75 ± 0.88)	11-13 (11.95 ± 0.50)
Pectoral	17-22 (19.25 ± 1.24)	18-24 (21.37 ± 1.59)
Gill rakers	28-38 (34.13 ± 2.47)	30-40 (35.25 ± 2.42)

Range with mean and SD in parentheses.

Table 3. Test of significance (ANOVA) for difference in meristic characters among the two localities.

Source of variation	df	MS	Fs
D1			
Among the groups	1	10.63	26.88***
Within the groups	138	0.3955	
Proportion of variation among groups (coefficient of intraclass correlation)		= 0.31	
D2			
Among the groups	1	11.71	19.60***
Within the groups	138	0.5974	
Proportion of variation among groups		= 0.25	
Anal			
Among the groups	1	1.921	2.97*
Within the groups	138	0.6471	
Proportion of variation among groups		= 0.33	
Pectoral			
Among the groups	1	129.02	70.62***
Within the groups	138	1.8269	
Proportion of variation among groups		= 0.55	
Gill rakers			
Among the groups	1	46.08	7.78**
Within the groups	138	5.9196	
Proportion of variation among groups		= 0.11	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

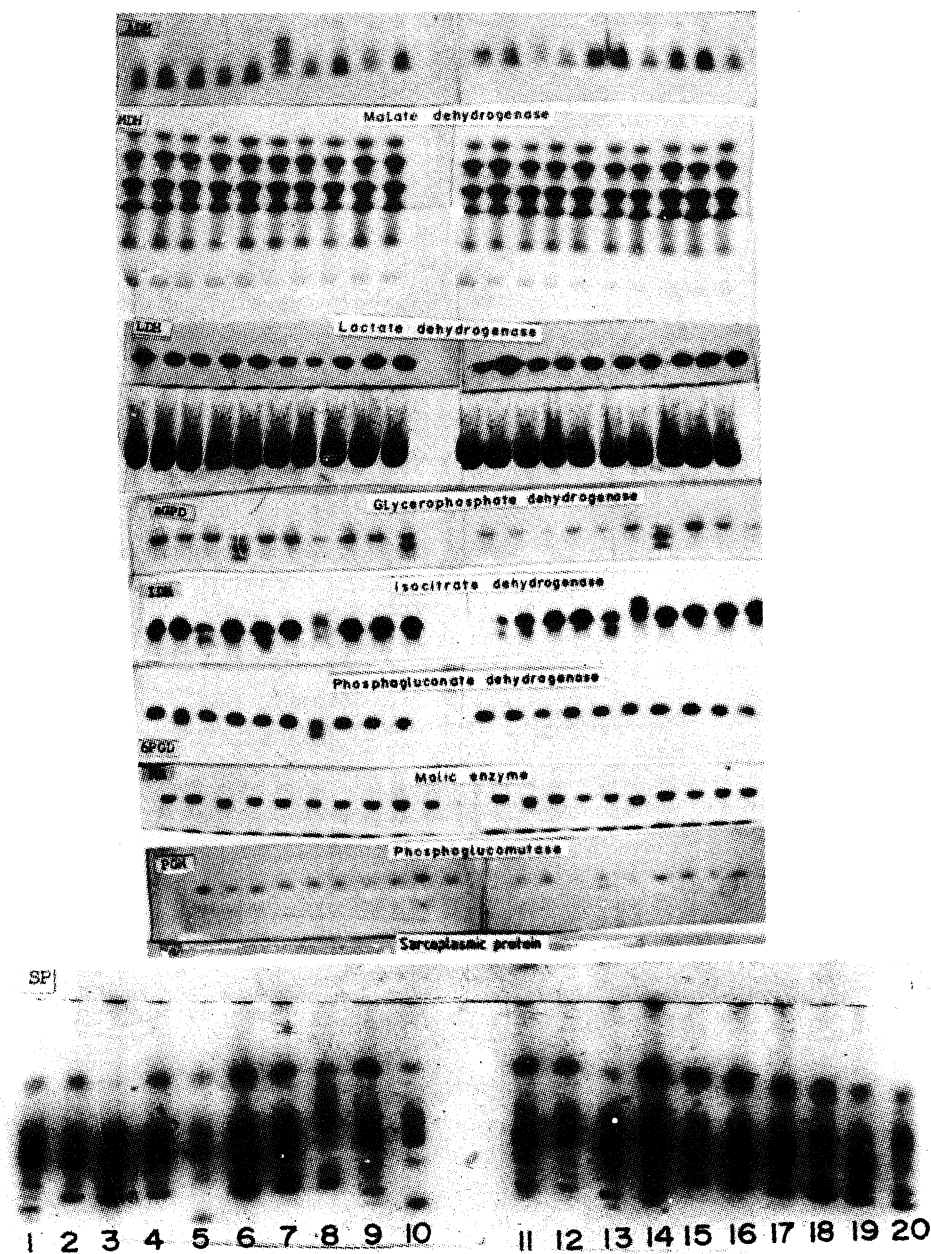


Figure 2. Gel photograph of isozyme patterns in 20 individuals of *R. kanagurta* from Panaji.

observed and expected numbers of phenotypes of each polymorphic enzyme are given in table 4. All of the loci except sp-2 were found to be in Hardy-Weinberg equilibrium. Significant departures from Hardy-Weinberg expectations were observed at sp-2 locus in both the localities (Panaji sp-2 $\chi^2 = 21.78$, $df = 1$, $P < 0.001$;

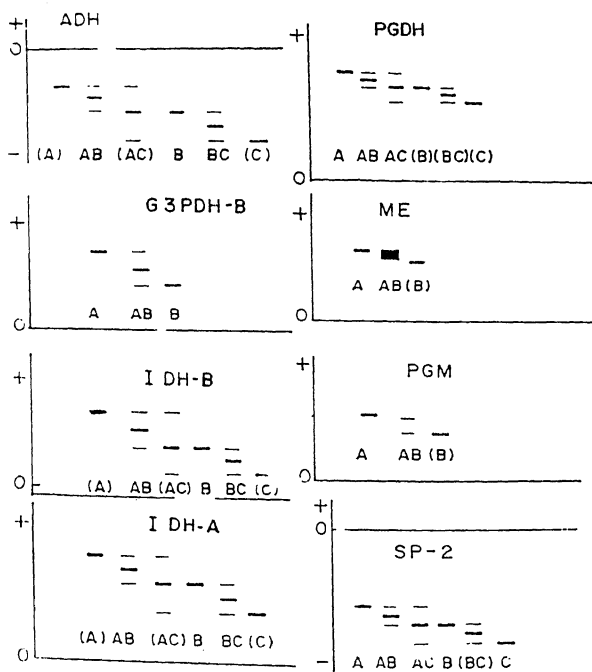


Figure 3. Diagrammatic representation of isozyme patterns of polymorphic enzymes in *R. kanagurta*, phenotypes in parentheses were not found.

Colva sp-2 $X^2=6.16$, $df=1$, $P<0.02$). The deviations reflected a deficiency of heterozygotes. Observed (H_o) and expected (H_e) heterozygosities at this locus were: Panaji $H_o=0.4$, $H_e=0.557$; Colva $H_o=0.325$, $H_e=0.384$. This locus showed the presence of 3 alleles (A, B and C) with most of the individuals being homozygous for sp-2 (A) allele (figure 2). A slightly significant heterogeneity was observed only at the sp-2 locus ($G_2=6.9$, $P<0.05$) between the two localities that may be due to chance (table 5).

The average number of alleles per locus was 1.59 (Colva) and 1.82 (Panaji), the proportion of polymorphic loci (P) using the 0.99 criterion of polymorphism (i.e. the frequency of the most common allele is less than or equal to 0.99) was 0.471 (Colva) and 0.529 (Panaji). At the 95% level of polymorphism the percentage of polymorphic loci was 17.6% (Colva) and 23.5% (Panaji). The average observed heterozygosity (H) per locus was 0.058 ± 0.098 (Colva) and 0.066 ± 0.110 (Panaji). A very low genetic distance (D) value was obtained between the two samples. The D value observed was 0.0017. This value lies in the lower range of the distribution of observed genetic distance values calculated between local populations of fishes which, in most cases, were not separated by as great a distance (Menezes *et al* 1990).

4. Discussion

The most informative estimate of genetic variation in natural populations is the average heterozygosity (H) per locus (Allendorf and Utter 1979). The amounts of P

Table 4. Allelic frequencies at polymorphic loci in *R. kanagurta* from two localities.

Locus	Locality	N	Allelic frequency			× 2
			A	B	C	
Adh	Panaji	80	0.075	0.919	0.006	0.626
	Colva	40	0.025	0.962	0.013	0.061
G3pdh-B	Panaji	100	0.875	0.125	0	2.041
	Colva	40	0.838	0.163	0	0.004
Hem	Panaji	40	0.975	0.025	0	0.026
	Colva	40	0.950	0.050	0	0.111
Idh-B	Panaji	78	0.025	0.968	0.006	0.086
	Colva	40	0.013	0.988	0	0.006
Idh-A	Panaji	60	0.008	0.967	0.025	0.007
	Colva	40	0.025	0.975	0	0.026
Me	Panaji	60	0.950	0.050	0	0.166
	Colva	40	0.975	0.025	0	0.026
Pgdh	Panaji	100	0.990	0.005	0.005	0.010
	Colva	40	1.000	0	0	—
Pgm	Panaji	100	0.990	0.010	0	0.010
	Colva	34	0.956	0.044	0	0.072
Sp-2	Panaji	60	0.600	0.242	0.158	21.78**
	Colva	40	0.760	0.175	0.063	6.16*

* $P < 0.05$; ** $P < 0.001$; N, number of individuals.**Table 5.** Heterogeneity test (using *G*-statistic) at the polymorphic loci in *R. kanagurta* between two localities.

Locus	<i>G</i>	<i>df</i>
G3pdh-B	0.665	1
Pgm	2.730	1
Me	0.829	1
Idh-B	1.320	2
Idh-A	3.940	2
Adh	2.990	2
Hem	0.706	1
Sp-2	6.91*	2

* $P < 0.05$.

and *H* vary non-randomly between loci, populations and species. In order to avoid a serious error in the estimation of average heterozygosity, a large number and wide range of isozyme loci should be examined (Allendorf and Utter 1979). The average heterozygosity values in *R. kanagurta* are more or less similar to the mean heterozygosity calculated by Nevo (1978) for 51 species of fishes ($H = 0.051 \pm 0.034$) and for 82 species of fishes ($H = 0.048 \pm 0.033$) calculated by Winans (1980). According to Nevo (1978) significantly larger heterozygosity values are found in tropical vertebrates that are ecological generalists (widespread, common, vagile, broad-niched mainland species). New genetic variation arises in a population from

either spontaneous mutation of a gene or by immigration from a population of genetically different individuals (Gooch 1975). The high amount of genetic variability as measured by isozyme loci is an indicator of high genetic variability throughout the genomes of this population. Populations with high average heterozygosities should also demonstrate high additive genetic variance for phenotypic traits which are of importance to fish culturists (Allendorf and Utter 1979).

The meristic characters of *R. kanagurta* showed that 67–89% of variation is within the groups and only about 11–33% is among the groups. The genetic basis for the variation of these characters is complex and they are also under the influence of numerous environmental factors (Ryman *et al* 1984). The significant variation within localities for these meristic traits may thus be evidence of different environmental conditions, to which the developing fish were subjected. Iwate (1973, 1975a,b) found morphological differences among groups of pollock in the sea of Japan and the Okhotsk sea, but found no differences in the frequencies of biochemical genetic variants. In all cases, the distribution of morphological variants most likely reflects regional environmental differences and does not necessarily reflect genetic subdivision (Iwate 1975b). Genetic differences between populations within a defined body of water may conceivably decrease with time, due to gene flow between subpopulations. Alternatively, genetic differentiation could become accentuated through time (perhaps because subpopulations become increasingly associated with discrete breeding areas) (Felley and Avise 1980).

Significant departures from Hardy-Weinberg expectations may be due to natural selection, migration or gene flow and genetic drift. We assume neutrality of allozymic variation to selection because it is a logical departure for testing hypothesis of genetic population structure (Grant *et al* 1987). Also in fish species with aggregate spawning and external fertilization the effect due to genetic drift is assumed to be nil, thus reducing the interpretation to: Are the patterns due to migration or gene flow from some other genetically differentiated populations? Further studies on the genetic structure of *R. kanagurta* from distant areas will no doubt further our understanding.

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Moulting patterns in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions

N SIVARAMI REDDY*†, T PAVAN KUMAR** and
K SASIRA BABU

Department of Zoology, S V University, Tirupati 517 502, India

Present address: *Central Sericultural Research and Training Institute, Srirampuram, Manandavadi Road, Mysore 570 008, India

**Central Silk Board, United Mansions, 39, M G Road, Bangalore 560 001, India

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Abstract. Moulting patterns in the silkworm, *Bombyx mori* L. (PM × NB4D2) under different photoperiodic conditions were studied. Moulting phenomenon, in *Bombyx mori* seems to be under the control of the circadian phenomenon. The peaks for the second moult were observed during the dark phase of the photoperiodic schedule expressing nocturnal predominancy. Consequently these peaks for consecutive moults moved into the light part of the 24 h cycle. This clearly demonstrates the instar dependency of the moulting expression. Under LL condition, a damp-out situation in moulting was observed. Moulting durations were greatly affected by photoperiods. Shortest moulting durations were recorded for LD 11:13.

Keywords. Moulting; photoperiods; *Bombyx mori*.

1. Introduction

Insect growth is characterised as discontinuous and is manifested by a series of moults. It is well established that the major features of the moulting cycles are regulated by a sequential action of 3 hormones; prothoracicotropic hormone (PTTH), ecdysone and juvenile hormone (JH) (Reynolds 1980; Happ 1984), the release and action of the PTTH is controlled by neurosecretory cells in the brain (Truman 1972). It is amply demonstrated that the optic lobes [the pupative biological clock(s) in insects] have a direct influence on the neurosecretory system (Page *et al* 1977). Photoperiodic effects on the timing of the moulting cycles can be accounted for on the basis of the neurosecretory system (Truman 1972; Beck 1980) being responsible for the secretion of PTTH, the first hormone in the moulting cycle sequence.

In our earlier communications (Sivarami Reddy *et al* 1984; Sivarami Reddy and Sasira Babu 1990), we have reported how the hatching, in *Bombyx mori* (L.) is influenced under different photoperiodic schedules. In the present paper, studies on the moulting in the commercial silkworm, *B. mori* (PM × NB4D2), under different photoperiodic conditions are reported.

2. Material and methods

Disease free layings (DFLs, each DFL consists of 350-450 eggs laid by a single silkworm on a single day) of the silkworm, *B. mori* (PM × NB4D2, a hybrid of Pure

†To whom all the correspondence should be addressed.

Mysore, a multivoltine and NB4D2, a bivoltine) were procured, third day after oviposition, for the study from the Government Silk Farm, Palamaner, Andhra Pradesh. The DFLs were transported to the laboratory during evening hours and immediately spread in the rearing trays. The same day, the DFLs were introduced, till completion of larval period, to normal day (LD 12:12), DD (continuous dark), LL (continuous light) and other different photoperiodic regimens, starting from LD 4:20 (short-day) to LD 20:4 (long-day). For all the photoperiodic combinations, the light phase (photophase) (around 50 lux) of the LD cycle commenced from 0600 h (for example for LD 12:12 condition the photophase was from 0600–1800 h and the scutophase spanned from 1800–0600 h). Optimum temperature ($25 \pm 1^\circ\text{C}$) and Rh ($80 \pm 5\%$) (Krishnaswami 1986) were maintained in the laboratory all through the experimentation. The experiment was conducted 5 times during different months in a year.

Two parameters in the moulting process of *B. mori*; one—the phase of settling for moulting (SM), when the larva stop feeding, with its thoracic segments bulging and fixing legs to the substratum adopting an 'S' shaped posture for moulting from which time the larvae are not supposed to be disturbed (Krishnaswami 1986), and the other parameter, the completion of moult (CM), the time at which the larva casts out its old cuticle and enters the next larval instar, were studied. Precise time (1 h interval) of SM and CM were recorded from all the (5) replications. Since the first instar larvae are very small in size for taking accurate observations, the observations on the first moult, both SM and CM, however, were not recorded. Further, the process for SM was already reported by us (Sivarami Reddy *et al* 1984) for two photoperiodic conditions; LD 12:12 and LD 11:13. The moulting duration, from SM to CM (between peak hours) for all the photoperiodic conditions was also computed based on the observations recorded for SM and CM.

3. Results

3.1 Moulting under LD 12:12 conditions

Second moult (both SM and CM), under LD 12:12 photoperiodic conditions recorded nearly 180° out of phase over hatching (Sivarami Reddy *et al* 1984; Sivarami Reddy and Sasira Babu 1990), with crests for SM and CM occurring at 20 h during the dark phase of the LD cycle (figures 1 and 5), thus expressing a nocturnal predominancy for the second moult. The peaks of SM and CM for third moult crested at 17 and 16 h respectively (figure 1). For fourth moult, the peaks for SM and CM crested at 13 and 9 h respectively. Thus successive moults (from II moult) crested gradually through the light phase of the LD cycle.

3.2 Moulting under continuous dark (DD) and light (LL) conditions

A definite rhythmic pattern in SM and CM has been observed under DD conditions for all 3 (II, III and IV) moults (figure 1). The trend in expression of the crests for consecutive moults from the second moult onwards was as that observed for LD 12:12 (figure 1). Thus the moulting peak of SM and CM for second moult was observed during late hours of the 24 h subjective cycle, showing a creeping

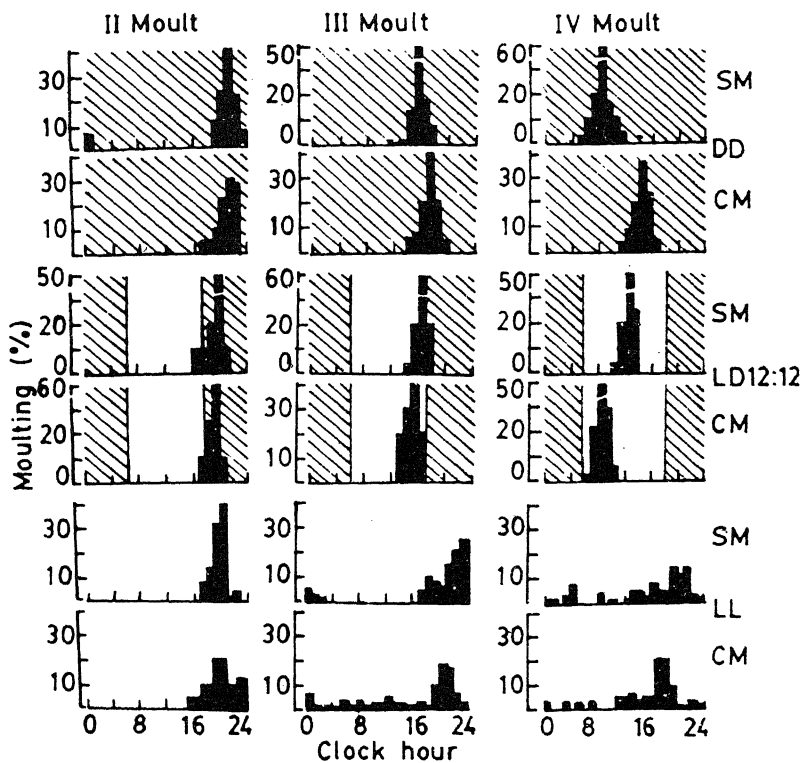


Figure 1. Moulting patterns in *B. mori* (PM×NB4D2) under natural solar day (LD 12:12), continuous dark (DD) and light (LL) conditions for the second, third and fourth moults. Note the nocturnal expression for the second moul shifting to a diurnal expression for the fourth moul through the third moul. Also, note the 'damp-out' expression in the moulting under LL conditions. Cross-hatched area represents the scutophase imposed.

phenomenon of crests for other successive moults into the first half cycle of the 24 h subjective cycle. However, the peak for the SM of fourth moul has been observed at 9 h while the same for CM continued expressing in the initial hours of the second half cycle.

Under continuous light (LL) conditions, the second moul was undoubtedly rhythmic (figure 1) with a distinct peak appearance. The expression of peaks for the successive moults too was rhythmic, however, with less defined peak appearance reminding a state leading to a damp-out expression. Except for the SM of the second moul, the quantum of larvae moulted at peak hour (observed under DD and LD) for all the other moults was not more than 20% (figure 1). For all the 3 moults studied, the moulting peak was observed during the second half of the 24 h day, predominantly after 16 h. Further, the moulting process was continuous spreading all through the 24 h day, the expression being more predominant for SM of fourth moul and CM of third and fourth moul.

3.3 Moulting under different photoperiodic conditions

Moulting under all the photoperiodic conditions was rhythmic, their peaks for the

second moult observed during the dark phase of the photoperiodic schedule and consequently these peaks for consecutive moults moved into the light part of the 24 h cycle (figures 2–4), thus indicating nocturnal expression for the initial moults, shifting to the diurnal expression for the later moults. This also clearly demonstrates the instar dependency of the moulting expression.

Under all the short-day conditions (LD 4:20 to LD 11:13), expression of the peak for SM (figure 2) has been observed between 18–22 h, thus during the early hours of imposed dark schedules, except for LD 11:13 condition where it was during late hours of the subjective day. The peak for CM also expressed a clear cut nocturnal predominancy for the second moult. Under long-day schedules (LD 13:11 to LD 20:4), the peaks for both the SM and CM were observed during the late hours of the imposed light schedules (figure 2), except for LD 13:11 and LD 14:10, for which these have been observed during early hours of the imposed night.

The crests for third moult (SM and CM) have been observed creeping towards light part of the 24 h subjective cycle (figure 3). Thus, under the short-day conditions, the moulting crests were observed during the early hours of the subjective night up to LD 9:15. For LD 10:14 and LD 11:13, these were observed

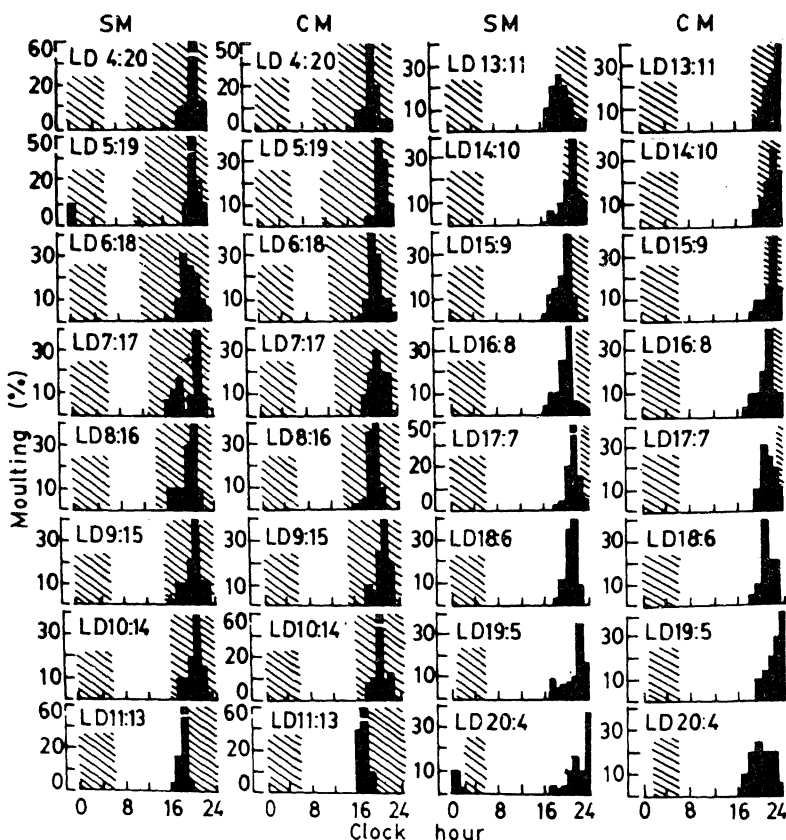


Figure 2. Moulting patterns of second moult in *B. mori* (PM×NB4D2) under different photoperiodic combinations. Note the nocturnal expression of moulting. Cross-hatched area represents the scutophase imposed.

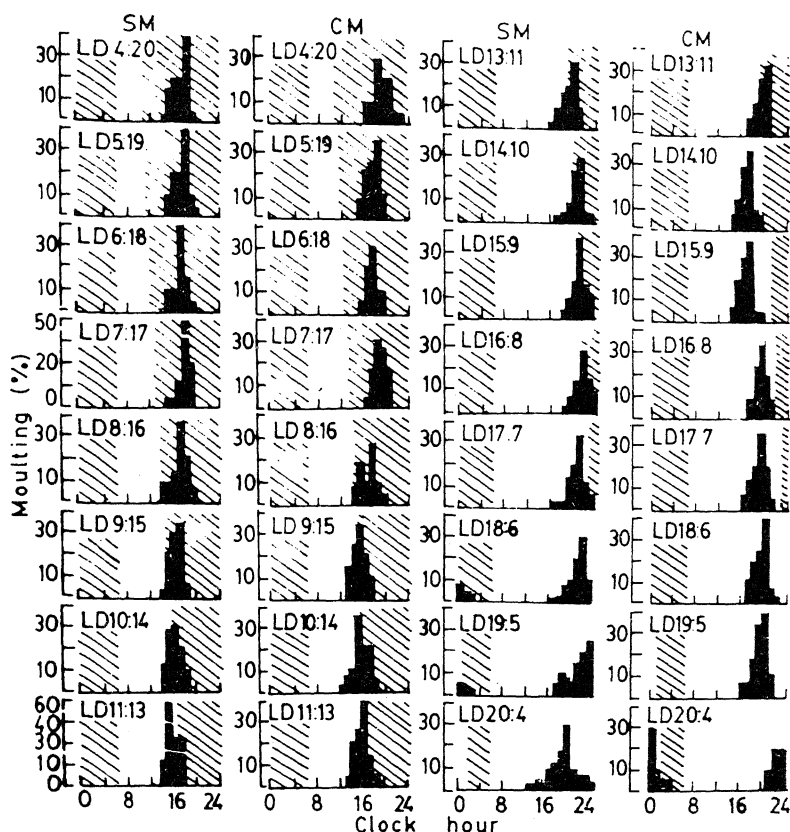


Figure 3. Moulting patterns of third moult in *B. mori* (PM×NB4D2) under different photoperiodic combinations. Note shifting of nocturnal expression in the second moulting towards light phase in third moult. Cross-hatched area represents the scutophase imposed.

during late hours of subjective day (figure 3). Also, under long-day conditions peaks of both SM and CM were observed during late hours of the imposed light schedules. Interestingly, the distribution time for SM under long-day conditions (figure 3), (LD; 18:6 to 20:4), was more over that of the other LD schedules showing a broadening tendency of the ecdysial distribution under (extreme) long-day conditions. However, the same for CM was comparable to that of the other photoperiodic regimes. Also under extreme long-day conditions, the moulting was confined to the late hours (20–24 h) of the 24 h cycle.

A distinct expression of the trend in shifting of the crests in moulting during subjective night hours for the second moult to a diurnal expression for the peak of fourth moult is clearly demonstrated, especially with the LD schedules very close to LD 12:12 (figures 4 and 5). In general, the peak moulting was observed during early hours (6–12 h) of the imposed light schedules under LD cycles close to LD 12:12 (figures 4 and 5). However, moulting was still nocturnal under short-day conditions from LD 5:19 to LD 8:16. Interestingly, for LD 4:20 the SM was observed during early hours of the imposed light part (figures 4 and 5), while the same for CM was during late hours of the subjective dark period indicating long

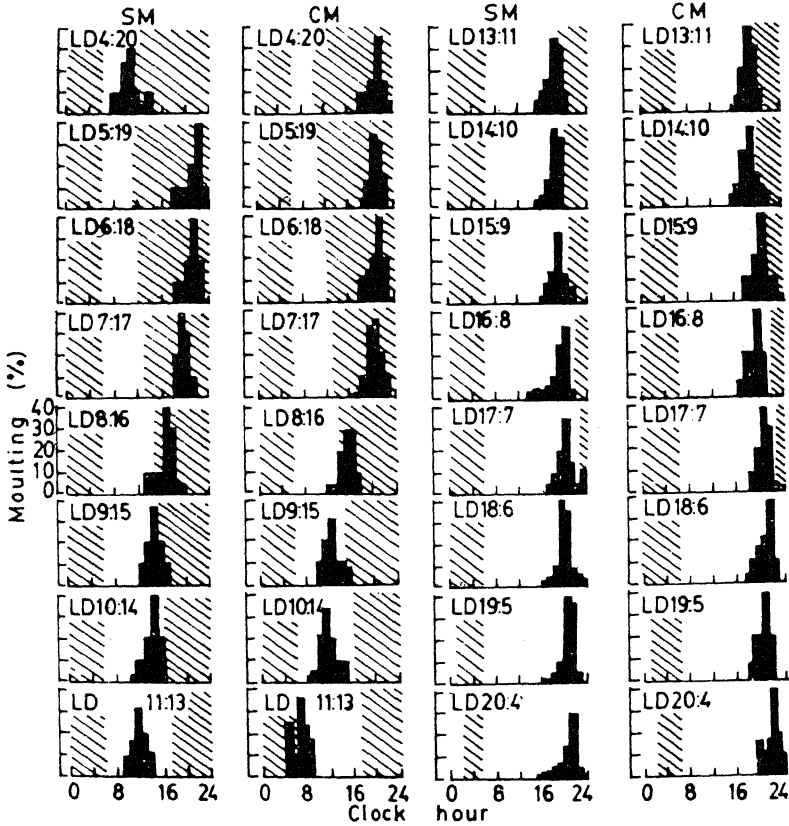


Figure 4. Moulting patterns of fourth moult in *B. mori* (PM \times NB4D2) under different photoperiodic combinations. Note the nocturnal expression of moulting in the second moult shifted to diurnal in the fourth moult, especially with the photoperiodic conditions close to LD 12:12. Cross-hatched area represents the scutophase imposed.

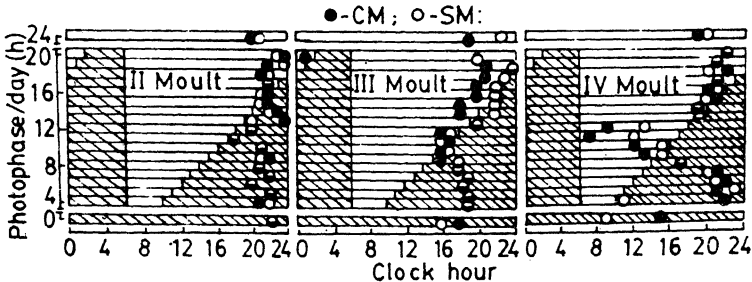


Figure 5. Expression of peak hours in moulting, in *B. mori* (PM \times NB4D2) under different photoperiodic combinations including LD 12:12, DD and LL. Note the nocturnal expression, in moulting for the second moult shifting to a diurnal component for the fourth moult through the third moult, especially with the photoperiodic combinations close to LD 12:12 (including LD 12:12). Each horizontal bar represents a photoperiodic combination in which peak hour of SM (○) and CM (●) are depicted. Cross-hatched area represents the scutophase imposed.

moulting duration (between peaks of SM and CM). Under long-day schedules, on the contrary, the moulting continued cresting during the late hours of the imposed light cycles. As observed for the third moult, the distribution time for SM, under extreme long-day conditions, was more compared to that under the other LD cycles.

3.4 Moulting durations

It is very interesting to note the effect of the photoperiod on the moulting duration, the time interval between the crests of SM and CM. Under extreme long and short-day conditions, the duration was observed to be more than 24 h while for LD conditions close to L 12:12, the same was around or less than 24 h (figure 6), thus indicating deferential implications of photoperiodism. Significantly, shortest moulting durations for all the 3 moults studied ($P < 0.05$), was recorded for LD 11:13 condition.

4. Discussion

Truman's (1972) extensive studies revealed that the larval ecdysis in *Antheraea pernyi* and *Manduca sexta* occur at a particular time of the day depending on species, the instar and the photoperiod. He reported that the larval moulting distribution time tend to broaden, in *A. pernyi* from initial instars to later ones. The first and second moults occurred during the light phase of both LD 12:12 and 17:7, but that of the third moult occurred in the following night and that of the fourth moult was delayed, occurring in the light phase again. A series of ligation experiments between the head and thorax at various times by Truman (1972) during the light cycles demonstrated that the release of the brain hormone (PTTH) was a gated phenomenon.

Beck (1980) pointed out that gated rhythms of larval (or nymphal) ecdysis have not been demonstrated and further this phenomenon has been considered by Beck (1980) as 'fortuitous synchrony' being the result of a gated rhythmic hormonal

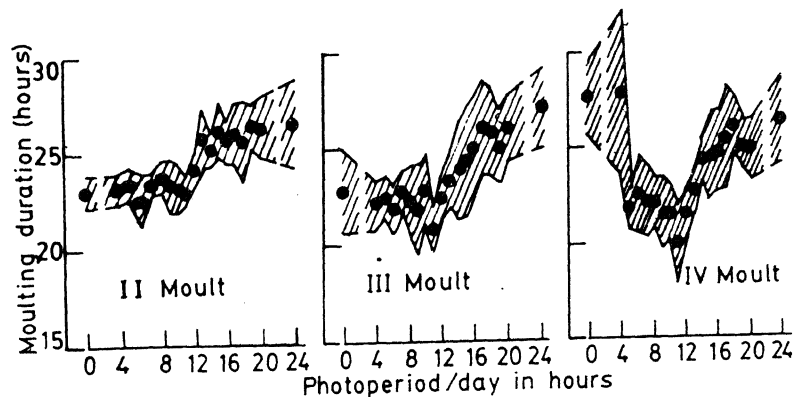


Figure 6. Moulting durations, from SM to CM (with deviations, represented by cross-hatched area) of second, third and fourth moults in *B. mori* (PM \times NB4D2) under different photoperiodic combinations. Note less moulting durations for short-day combinations, especially LD 11:13.

release (Truman 1972). Thus, the larval ecdysis themselves were not gated, however, but occurred after a fixed number of hours of hormonal (PTTH) release (Saunders 1982).

The observed patterns of rhythmicity in larval ecdysis, in the present study, the SM and CM (figure 1) under LD 12:12 conditions and the persistence of the LD 12:12 pattern under all the other photoperiodic conditions including the continuous conditions (figures 1–5) suggests the endogenously free-running nature. Moreover, a damp-out (as less defined peak expression observed) stage of moulting expression has been observed under LL condition (figure 1), especially for third and fourth moults, supporting the above observation. It has amply been demonstrated that the eclosion rhythm in *Drosophila pseudoobscura* (Pittendrigh and Bruce 1957; Chandrashekaran and Lohar 1969) showed a fairly rapid damping out to eventually arrhythmicity under LL conditions. Saunders (1982) stated that although the eclosion may be aperiodic in LL, a rhythm becomes apparent after transfer of the culture from LL to darkness (Winfree 1970, 1972). Thus the present results on moulting seems to be of gated in nature (after fixed hours of PTTH release, a subject for further probe). Beck (1980) viewed that, though the experimental data of Truman (1972) strongly suggests that the gating rhythm was circadian, its endogenous nature was not actually demonstrated. Most significantly, Truman (1972) found that though the release of PTTH is a gated event, the secretion of growth hormones (ecdysone and JH) coming into act, subsequent release of PTTH in the moulting cycle were not gated. However, the synchrony of larval ecdysis appeared to be dependent solely on the gated secretion of PTTH. In the present study, the synchrony of the CM appears to be dependent on the SM. However, the question whether the SM in *B. mori* is the immediate response of PTTH hormonal release or not would be an interesting subject for further research.

Anantha Narayana *et al* (1978) demonstrated that in multivoltine (Pure Mysore) *B. mori* the light-on signal is taken as reference point in rhythms of eclosion and egg hatching while light-off for oviposition rhythm. For bivoltines, they reported that light-off is taken as reference point for eclosion rhythm and neither on or off for oviposition rhythm. Sivarami Reddy and Sasira Babu (1990) also reported light-on as a possible reference point for egg hatching in *B. mori*. For larval moulting, however, neither light-on nor off seems to be taken as a reference point. However, the peak moulting expression, in the present study seems to be instar dependent as reported for *A. pernyi* and *M. sexta* (Truman 1972) which was attributed to the differences in duration of successive stadia or instars. Krishnaswami (1986) had clearly reported significant variations in the durations of different larval stadia in *B. mori*.

The time intervals between the release of hormone (PTTH) and the completion of larval ecdysis is again reported as instar specific and temperature dependent (Truman 1972; Beck 1980; Saunders 1982) as Truman (1972) demonstrated a 3°C raise in the temperature from 25–28°C resulted in 2 h advancement of the moult. Since the larvae of *B. mori*, in the present study, were maintained at a constant temperature (25 ± 1°C), the duration seems to be influenced by photoperiod as also the instar, as suggested by Truman (1972).

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Effect of insect growth regulators on hatching of eggs of three vector mosquito species

V VASUKI

Vector Control Research Centre (ICMR), Pondicherry 605 006, India

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Abstract. Hatchability of eggs of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* was studied by exposing freshly laid (0-1 h) and older (12-18 h) eggs to varying concentrations (0.0001-1.0 mg/l) of 6 insect growth regulators viz. OMS 3019, OMS 3007, OMS 2015, OMS 3031, OMS 3013 and OMS 3009. In all the 3 species, dosage dependent response was observed. The response was also dependent on the age of eggs. Among the 6 IGR compounds OMS 3031 was found highly active resulting in maximum reduction in hatching of freshly laid eggs of *Anopheles stephensi* (99.95%) followed by *Aedes aegypti* (89.9%) whereas 50% inhibition in hatching occurred in older eggs of *Culex quinquefasciatus* exposed to OMS 3009 at the same dosage. Dose, age and dose and age dependent hatching was evident in eggs of the 3 test species exposed to insect growth regulators OMS 3019, OMS 3013 and OMS 2015. Higher proportion of unhatched eggs with varying abnormalities was noticed in test species. Percentage mortality observed was higher in first instar larvae hatched from treated eggs reared in untreated water. Therefore, insect growth regulators have great potency in suppressing the population by affecting hatching of mosquito eggs in addition to inhibition of adult emergence, thus providing a useful tool for integrated vector management.

Keywords. Insect growth regulators; egg hatchability; post-treatment effects; *Culex quinquefasciatus*; *Aedes aegypti*; *Anopheles stephensi*.

1. Introduction

Insect growth regulators (IGRs) have received a great deal of attention in the last two decades as promising insect control agents (Retnakaran *et al* 1985) because of their unique mode of action of disrupting the metamorphic development, higher selectivity and less persistence in the environment (Post and Vincent 1973; Mulder and Gijswijt 1973). In addition, many new IGRs currently under development have low mammalian toxicity and are potentially compatible with natural enemies and safer to economically important insect species (Staal 1975). Such attributes are desirable when dealing with problems of vector resurgence, secondary vector outbreaks and insecticide resistance. Thus, IGRs can be effectively used in integrated vector management (IVM) programme designed to decelerate or prevent the development of resistance (Sparks and Hammock 1982).

IGRs, not only interfere with metamorphosis or chitin deposition during moulting (Mulder and Gijswijt 1973; Staal 1975; Sparks and Hammock 1982; Estrada and Mulla 1986), but also affect the hatching of eggs of medically and agriculturally important insects (Chokalingam and Noorjahan 1984; Jordan *et al* 1979; Saxena and Girish Mathur 1981; Ascher and Nemny 1974; Moore and Taft 1975). However, very little information is available on the effect of IGRs on hatching of mosquito eggs.

The present study is undertaken to determine the effect of 6 IGRs on hatching of

eggs of 3 major vector mosquitoes, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*.

2. Materials and methods

Six new IGRs (4 chitin synthesis inhibitors and two juvenoids) were used for this study. Four chitin synthesis inhibitors are OMS 2015 (Triflumuron, 1.04% gr), OMS 3009 (CME 13406, 15% water based suspension concentrate), OMS 3031 (XRD-473, 5% ec) and OMS 3013 (IK 17899, 5% ec) and two juvenoids are OMS 3019 (S31183, 5% wp) and OMS 3007 (S21149, 5% wp). For each compound 1% stock solution was prepared and serial dilutions were made thereafter. The test species of mosquitoes *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were obtained from cyclic colonies maintained in Vector Control Research Centre.

Newly emerged mosquitoes were held in 30 × 30 cm netted cage provided with raisin and 10% glucose solution. They were offered blood meal from a chicken or rabbit as the case may be, on the third day after emergence. Oviposition cups were provided after 3 days. In this way 12–18 h old eggs were obtained. A single egg raft of *C. quinquefasciatus* and 100 eggs of *Ae. aegypti* and *An. stephensi* were exposed to IGR treated waters with concentrations ranging from 0.0001–1.0 mg/l. In another set of experiments individual gravid females were allowed to oviposit in small cups (50 ml) gauzed with nylon net. Thus, freshly laid eggs were exposed to IGR treated water within 1 h. Four replicates for each concentration and appropriate controls were maintained. Hatchability observations were made after 36–48 h of incubation period and abnormalities in hatched and unhatched eggs were recorded. The larvae hatched from treated (1.0 mg/l) eggs were reared in treated and untreated water for the observation on post embryonic development.

Analysis of variance was performed using the ANOVA procedures (Sokal and Rohlf 1981) depending on whether the experiment involved a balanced or unbalanced design. The data from the egg hatching studies, expressed as percentages, were transformed to arcsin angular transformation to normalize the distribution before testing by ANOVA. This procedure was used because the comparison wise error rate was considered to be more important than the experiment-wise error rate. This allows all possible comparisons between all experimental groups.

3. Results and discussion

Figure 1 summarizes the level of inhibition in hatching of eggs (12–18 and 0–1 h old) of *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* exposed to 6 IGRs mentioned above at series of concentrations. On screening the effect of these IGRs it is evident that the hatching of treated eggs at dosages ranging from 0.0001–1.0 mg/l is greatly reduced. On the basis of percentage inhibition in hatching of eggs (0–1 and 12–18 h old) of the three test species at the maximum dosage, the compounds can be compared in the order of their efficacy. OMS 3031 was found to have significantly highest efficacy causing maximum suppression in hatching in (0–1 and 12–18 h old) eggs of *Ae. aegypti*, 12–18 h old eggs of *C. quinquefasciatus* and 0–1 h old eggs of *An. stephensi* while OMS 3009 was more active against 0–1 h old *C. quinquefasciatus* and 12–18 h old *An. stephensi*.

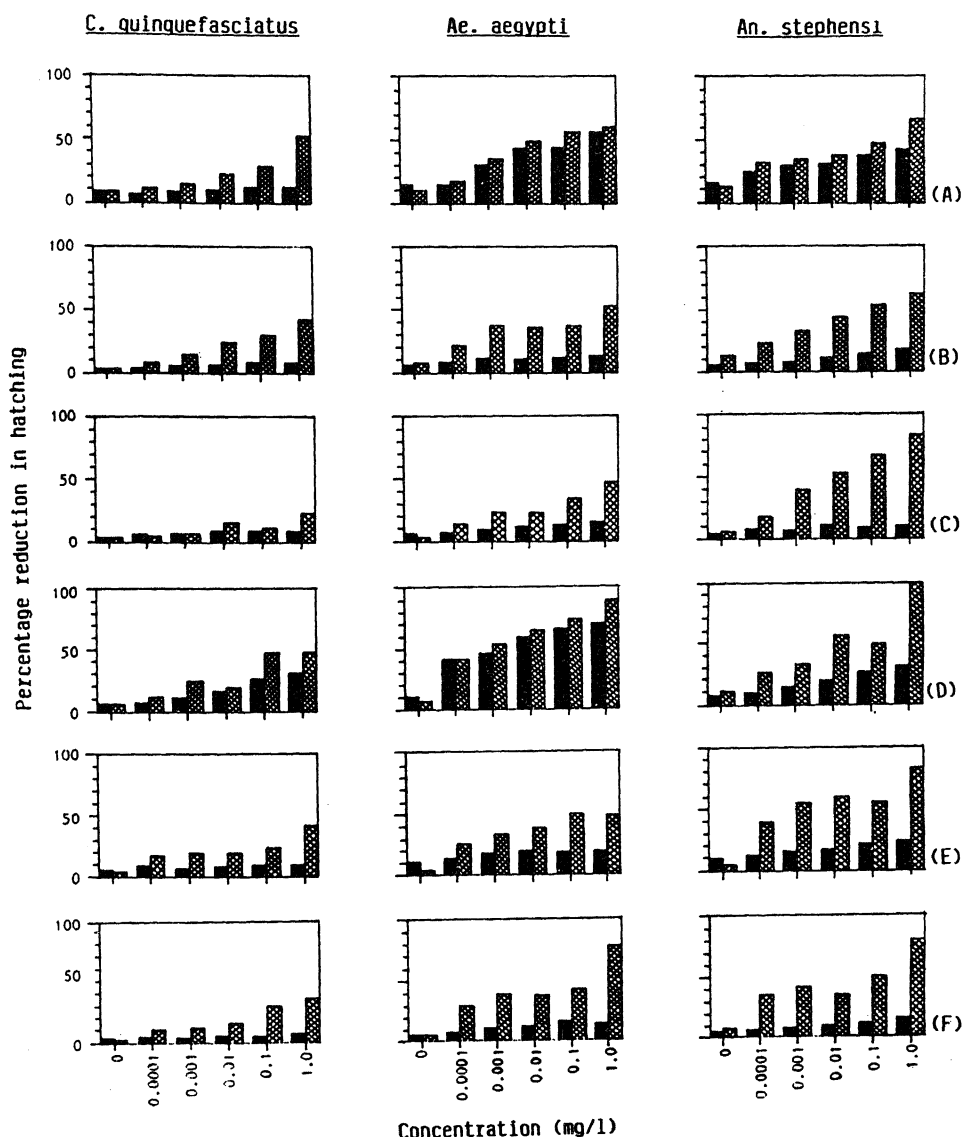


Figure 1. Percentage reduction in hatching of 12-18 h old (■) and 0-1 h old (▨) eggs of *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* exposed to 6 IGRs. (A), OMS 3009; (B), OMS 3007; (C), OMS 2015; (D), OMS 3031; (E), OMS 3013; (F), OMS 3019.

The results show that the reduction in hatching of mosquito eggs is significantly related to dosage ($P < 0.05$) in all cases. However, significant age dependence could only be demonstrated in the case of *C. quinquefasciatus* (for all the IGRs). Dose and age dependence could be observed in all the 3 species tested only for 3 IGRs (OMS 3019, OMS 3013 and OMS 2015).

The overall response in the eggs of all test species varied at different concentrations of treated waters showing more unhatched eggs with various abnormalities at increasing concentrations. When the eggs of 3 species were exposed

to 6 IGRs younger embryos-preblastoderm (Idris 1960) were found to be more sensitive than older ones suggesting the influence of the age of the embryos at the time of treatment on the activity of the candidate IGR compounds at increasing dosages. Similar inverse relationship between the age of the egg and the susceptibility to chitin synthesis inhibitor, diflubenzuron has been observed in Egyptian cotton leaf worm (Ascher and Nemny 1974), Boll weevil (Moore and Taft 1975), Simulium (Lacey and Mulla 1978) and Southern house mosquito (Miura *et al* 1976).

Percentage reduction in hatching is found to be higher in eggs exposed to chitin synthesis inhibitors than eggs exposed to juvenoids irrespective of the species and age of the eggs.

Typical symptoms of toxicity and developmental abnormalities induced by IGRs on exposure of mosquito eggs (both younger and older) have been observed and are as follows.

Unhatched eggs contained fully developed embryos which failed to hatch and apparently died just before hatching. Segmentation, eye spots, eggspine and setae were visible through the eggshell of *C. quinquefasciatus*.

In some cases larvae eclosed from a longitudinal line of weakness at the mesal dorsum of eggshell which is different from normal hatch where a portion of the anterior end of the egg shell is forced open transversely at a line of dehiscence, forming an egg cap which is not completely detached but hinged to the remaining egg shell in the case of *C. quinquefasciatus* and *An. stephensi*. Partial side hatch resulted in the death of the larva during ecdysis. Larvae were found dead with (i) head capsule free, but caudal end still caught in the egg shell, (ii) caudal end free but head capsule inside the egg shell and (iii) thorax and abdomen free but head capsule and caudal end in egg shell. IGR treatment may also have caused slower embryonic development as reported earlier in *C. pipiens quinquefasciatus* (Miura *et al* 1976).

These compounds may affect the chitin deposition in cuticle thus the rigidity of the cuticle is of lower degree and it fails to resist the muscular traction during hatching thereby resulting in the death of the larvae within the eggs as observed in *Spodoptera littoralis* treated with diflubenzuron (Ascher and Nemny 1974). It is also possible that these compounds interrupt the development shortly before hatching making the heads of the larvae to be seen outside the egg shell as suggested by Saxena and Girish Mathur (1981).

The first instar larvae hatched from eggs treated with OMS 3031 and OMS 3009 showed 100% mortality in the case of *C. quinquefasciatus* followed by 94–96% in *Ae. aegypti* and 72–98% in *An. stephensi*. Whereas, 54–90% mortality was observed in first instar hatched from the eggs treated with the other compounds (OMS 3007, OMS 3019, OMS 2015 and OMS 3013) in the 3 species tested (tables 1–3). High mortality recorded in first instar larvae hatched from IGR treated eggs (1 mg/l) reared in untreated water may be due to the transfer of the compound from egg to larva as reported earlier (Saxena and Girish Mathur 1981). Complete inhibition of adult emergence resulted in the larvae of the 3 test species hatched from the eggs treated with OMS 3031, OMS 3009 and OMS 3013. Only 6–14%, 4–41% and 8–10% adult emergence was recorded in *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively as an indication of post treatment effect on the larvae hatched from eggs treated with 3 IGRs (OMS 3007, OMS 2015 and OMS 3019). However,

Table 1. Post-treatment effects of IGRs on the larvae of *C. quinquefasciatus* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%)				Pupation (%)	Pupal mortality (%)	Adult emerged (%)
	I	II	III	IV			
OMS 3019	62(3.6)	22(2.2)	10(1.0)	—	6(1.0)	—	6(1.0)
OMS 3007	60(5.1)	10(2.2)	8(2.8)	2(1.0)	20(4.4)	6(1.0)	14(3.6)
OMS 2015	76(1.4)	2(1.0)	—	—	22(1.0)	10(2.2)	12(2.8)
OMS 3031	100(0.0)	—	—	—	—	—	—
OMS 3013	79(3.0)	19(2.6)	2(1.0)	—	—	—	—
OMS 3009	100(0.0)	—	—	—	—	—	—
Control	6(1.0)	2(1.0)	—	2(1.0)	90(1.0)	—	84(2.0)

Values are mean \pm SE.**Table 2.** Post-treatment effects of IGRs on the larvae of *Ae. aegypti* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%)				Pupation (%)	Pupal mortality (%)	Adult emerged (%)
	I	II	III	IV			
OMS 3019	4(3.2)	18(2.2)	14(2.2)	—	4(1.4)	—	4(1.4)
OMS 3007	54(5.4)	3(0.9)	—	—	43(5.1)	2(1.0)	41(4.3)
OMS 2015	60(4.5)	6(1.0)	4(1.4)	—	30(2.2)	2(1.0)	28(3.1)
OMS 3031	94(2.2)	6(2.2)	—	—	—	—	—
OMS 3013	90(2.2)	8(2.8)	2(1.0)	—	—	—	—
OMS 3009	96(1.4)	4(1.4)	—	—	—	—	—
Control	2(1.0)	4(1.4)	10(4.1)	2(1.0)	82(5.0)	2(1.0)	80(4.2)

Values are mean \pm SE.**Table 3.** Post-treatment effects of IGRs on the larvae of *An. stephensi* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%)				Pupation (%)	Pupal mortality (%)	Adult emerged (%)
	I	II	III	IV			
OMS 3019	58(3.6)	6(2.2)	12(4.5)	4(1.4)	20(4.4)	10(3.0)	10(2.2)
OMS 3007	70(2.2)	1(0.9)	12(1.4)	6(2.2)	10(2.2)	2(1.0)	8(1.4)
OMS 2015	60(4.2)	—	12(4.5)	6(2.2)	22(2.2)	12(0.0)	10(2.2)
OMS 3031	98(1.0)	2(1.0)	—	—	—	—	—
OMS 3013	64(5.8)	12(2.4)	24(2.8)	—	—	—	—
OMS 3009	72(4.5)	28(3.2)	—	—	—	—	—
Control	3(1.7)	2(1.0)	6(1.0)	—	88(2.4)	1(0.8)	87(2.1)

Values are mean \pm SE.

large proportion of the larvae hatched from abnormal hatching or normal hatching died during next ecdysis and all the larvae were dead within 48 h in treated waters.

Besides being potent chitin synthesis inhibitors and disruptors of metamorphic development causing higher mortality in immatures, IGRs are also found to greatly affect hatching of mosquito eggs soon after oviposition or just before hatching.

Therefore, IGRs true potentiality is much higher than the anticipated inhibition in adult emergence as often indicated by EI_{50} values. Hence, the significant role of IGRs in preventing the hatching of mosquito eggs may be included in the evaluation as their promise in integrated vector control is obvious.

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A feeding test to identify rice varieties resistant to the leaf folder, *Cnaphalocrocis medinalis* (Guenee)

J S BENTUR and M B KALODE

Directorate of Rice Research, Rajendranagar, Hyderabad 500 030, India

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Abstract. Dynamics of leaf feeding by the larvae of rice leaf folder *Cnaphalocrocis medinalis* (Guenee) was studied under greenhouse conditions to develop a feeding test to identify varietal resistance. Area of leaf damaged by larvae during their development indicated that first 3 instars accounted for only 8.06% and V instar alone for 66.3% of the total feeding. In view of the highest feeding rate and the longest feeding duration, V instar was chosen for feeding test. No significant differences in area of leaf damaged were recorded when V instar larvae fed for 48 h on 30, 45 and 60 days old plants or when leaf nitrogen content varied from 2–3.4%. Inherent variability among individual larvae in feeding rate could be maintained within acceptable limits with 5 replications.

The proposed feeding test involved caging of individual V instar larvae for 48 h on 30–45 days old plants of test varieties and recording area of leaf damage. The test revealed varietal differences in area of leaf damaged by the leaf folder and displayed consistency over time. Based on feeding test, 19 rice varieties have been identified as resistant against leaf folder.

Keywords. Rice leaf folder; feeding test; varietal resistance; *Cnaphalocrocis medinalis*.

1. Introduction

The rice leaf folder, *Cnaphalocrocis medinalis* (Guenee), is an important pest in almost all of the rice growing countries of Asia. Pest outbreaks and consequent damage to the crop have been reported from Japan (Wada *et al* 1980), Nepal (Pradhan and Shahi 1983) and other countries (Heinrichs *et al* 1985). In India, severe damage due to this pest has been recorded from Maharashtra (Dorge *et al* 1971), Madhya Pradesh (Gargav *et al* 1971), Orissa (Yadava *et al* 1972), Gujarat (Upadhyay *et al* 1975), West Bengal (Chatterjee 1979), Uttar Pradesh (Verma *et al* 1979) and Haryana (Ram 1986).

Economic injury to the crop is related to the extent of leaf damage and period during crop growth when the damage is inflicted (Bautista *et al* 1984; Miyashita 1985). Varietal differences in the extent of damage by the leaf folder have been extensively noted based mainly on the natural incidence and leaf damage, as feeding is principally restricted to green mesophyll tissue (Fraenkel *et al* 1981). Non-uniform pest pressure and unpredictable buildup limit field evaluation as a reliable technique to assist host-plant resistance breeding programmes. As an alternative, greenhouse screening of rice varieties has been suggested by Yadava *et al* (1972), Waldbauer and Marciano (1979) and Heinrichs *et al* (1985). These methods, generally require prolonged rearing of the pest on test entries and recording insect survival and leaf damage. On the other hand, Das and Nair (1974) outlined a simple method of directly recording area of leaf feeding by individual larvae. In the

present studies, we have attempted to validate this approach by studying dynamics of leaf feeding by *C. medinalis* larvae on selected test varieties and propose a feeding test to rapidly identify resistant varieties in the greenhouse.

2. Materials and methods

2.1 Rearing of the insect

The rice leaf folder, *C. medinalis*, is being reared in the greenhouse at the Directorate of Rice Research since 1979. Larvae were reared on rice variety, TN 1 (susceptible) while adults were provided with 10% sucrose solution. Eggs were collected by caging 10–15 pairs of adults on 20–25 days old potted rice plants. Temperatures in the greenhouse ranged from 20–36°C and humidity 50–85% RH. Once in a year, field collected adults were mixed with the greenhouse reared insects to avoid inbreeding.

2.2 Measurement of larval feeding

Strips of leaves with black head stage eggs were kept in Petri-dishes containing wet cotton wool. Neonate larvae (25) from these were caged individually at 1 larva/plant on 30 days old plants. Larval survival and moultings were noted. Larvae were transferred daily to new plants. Width of head capsule in each instar was measured with an ocular micrometre under a 30X binocular microscope. Area of leaf damaged by the larva was measured by holding the leaf against mm² graph paper and counting the mm squares visible through the translucent scraped area when held against light. Means were computed to determine the amount of feeding on successive days and during specific larval instars.

2.3 Factors influencing leaf feeding

2.3a Plant age: Plastic pots containing 30, 45 and 60 days old plants of TN 1 were selected for this study. Fifth instar larvae were individually caged on these plants for 48 h. Area of leaf damage was measured as described earlier. Replications for each age group were 7 or 8.

2.3b Level of nitrogen in leaf: Potted TN 1 plants were raised with and without nitrogen fertilization. In nitrogen applied pots urea top dressing was given at 100 kg N/ha in two equal splits on 5 and 20 days after sowing. For each level of fertilizer application, 5 pots each containing ten 30 days old plants were used. Fifth instar larvae were individually caged on each set of plants for 48 h. Prior to release, only mother tillers of 5 plants per pot were retained by cutting off other tillers. Second and third leaves from the top of the primary tillers of the remaining 5 plants were pooled for estimating nitrogen after oven drying. While 13–17 replications were maintained for estimating leaf damage by larvae, 3 replications were kept for nitrogen estimation by micro kjeldahl digestion method.

2.4 Variability in leaf feeding

Several sets of observations over a period of 24 months, each with varying number

of replications were considered to note the variability in amount of leaf feeding in different batches of V instar larvae. Each observation consisted of caging one larva on 30–45 days old TN 1 plants for 48 h and recording leaf area damaged. Variability was expressed in terms of SE as per the formula:

$$\text{Variability index} = \frac{\text{SE}}{\text{Mean}} \times 100.$$

Based on this index, the optimum number of replications was determined.

2.5 Varietal evaluation

On the basis of results obtained in the above investigations, rice varieties were evaluated by caging V instar larvae individually on 30–45 days old plants of test varieties for 48 h. Damaged leaf area was measured at the end of this period as described. Varieties were screened in sets of 5–10 entries along with the susceptible check TN 1. After initial sets of results, PTB 12 was used as resistant check. Four to ten replications were maintained. Average values of leaf area damaged in test entries were statistically compared with that for susceptible check variety in the corresponding set. After several sets of screening, consistency in values against susceptible and resistant checks was tested.

3. Results

3.1 Measurement of larval feeding

Area of leaf scraped by larvae on each successive day is presented in figure 1. While 10 of the initial 25 larvae survived till pupation, active feeding was observed up to

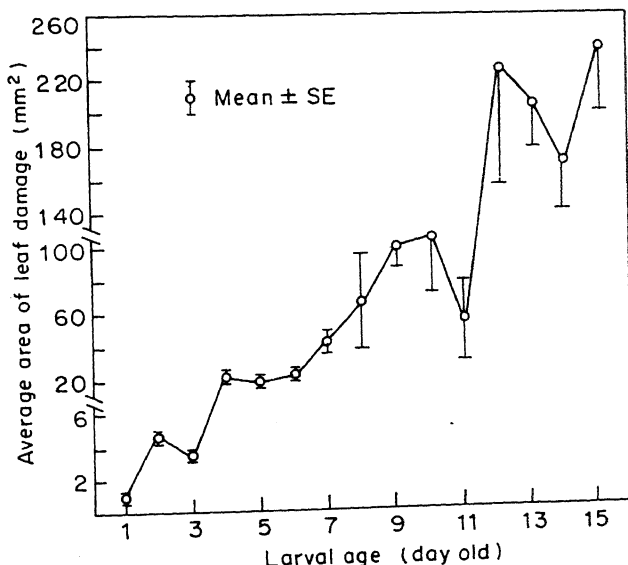


Figure 1. Leaf area damage in susceptible TN1 rice variety by the leaf folder, *C. medinalis* larvae.

15 days. All larvae underwent 5 instars. Area of leaf damaged by each of the 5 instars, duration of larval instars along with width of the head capsule is given in table 1. It is evident that 3 initial larval instars accounted for only 8.06% of the total area of the leaf fed, while V instar alone contributed to 66.3% of the total feeding. Width of the head capsule in each instar registered distinct difference and instars could be visually identified. It is thus logical to expect any differences in the amount of feeding by larvae to be clearly manifested when V instar larvae are involved.

In another study, neonate larvae during a period of 5 days did 4.2% of total feeding, while 10 days old larvae accounted for 69.7% (table 2). These results generally agreed with the earlier study since 10 days old larvae tended to be in V instar and these instars fed most voraciously. Based on these experiments only V instar larvae were used in the subsequent studies.

3.2 Factors influencing leaf feeding

3.2a Plant age: Leaf area damaged by V instar larvae in 48 h when caged on 30, 45 and 60 days old TN 1 plants is given in table 3. Though slightly higher amount of feeding was noted on 30 days old plants, no significant differences were evident with respect to plant age in the range tested.

3.2b Level of nitrogen in leaf: Leaf feeding by V instar larvae on TN 1 plants raised under two different levels of nitrogen fertilizer is presented in table 4. Though level of nitrogen in leaf in these two sets of pots was significantly different from each other, leaf area damaged by the larvae did not differ significantly.

Table 1. Width of head capsule, duration and leaf area scraped by different instars of *C. medinalis*.

Larval instar	Width of head capsule (mm)	Larval duration (days)	Leaf area scraped (mm ²)	Average leaf area (mm ²)	Percentage of total area scraped
	Mean \pm SE	Mean \pm SE	Mean \pm SE	scraped/day	
I	0.23 \pm 0.001 (10)	2.29 \pm 0.18	7.06 \pm 1.24 (23)	3.08	0.59
II	0.36 \pm 0.002 (15)	2.14 \pm 0.14	30.11 \pm 4.96 (18)	14.07	2.50
III	0.53 \pm 0.006 (11)	2.20 \pm 0.20	59.44 \pm 10.82 (15)	27.01	4.97
IV	0.81 \pm 0.007 (10)	3.75 \pm 0.25	306.00 \pm 68.30 (13)	81.60	25.60
V	1.06 \pm 0.013 (10)	5.25 \pm 0.25	793.00 \pm 123.80 (11)	151.05	66.32
Total	—	15.00 \pm 0.40	1195.6	—	100

Numbers in parentheses are replications.

Table 2. Leaf feeding by different aged larvae of *C. medinalis*.

Age of larvae	Leaf area scraped in 5 days (mm ²)	Total feeding (%)
	Mean \pm SE	
Neonate	46.9 \pm 7.5 (10)	4.2
5 days old	449.8 \pm 28.7 (10)	26.1
10 days old	807.0 \pm 63.4 (10)	69.7

Numbers in parentheses are replications.

Table 3. Influence of plant age on leaf feeding by V instar *C. medinalis* larvae.

Plant age	No. of replications	Leaf area scraped in 48 h (mm ²)
		Mean \pm SE
30 days old	7	298.8 \pm 35.7 ^a
45 days old	8	276.0 \pm 21.5
60 days old	7	211.3 \pm 25.8 ^b

Comparison of means (*t* test). *a*-*b*, Not significant.

Table 4. Influence of level of nitrogen in leaf on feeding by V instar *C. medinalis* larvae.

Treatment	Level of nitrogen in leaf (% dry wt.)	Leaf area scraped in 48 h (mm ²)
	Mean \pm SE	Mean \pm SE
Low nitrogen*	2.00 \pm 0.17 ^a (3)	242.2 \pm 30.5 ^c (13)
High nitrogen**	3.40 \pm 0.06 ^b (3)	254.5 \pm 28.5 ^d (17)

Comparison of means (*t* test): *a*-*b*, $P < 0.01$; *c*-*d*, Not significant; Numbers in parentheses are replications.

*Plants raised in puddled soil with no application of N fertiliser.

**Plants raised with two top dressings of N at 50 kg N/ha on 5th and 20th day after sowing.

3.3 Variability in leaf feeding

Data of several sets of observations with 4-12 replications indicated leaf feeding variability index to be negatively correlated with the number of replications ($r = -0.959$). It could be computed from this regression that 5 replications would result in 11.4% variability, whereas in practice sets of 5 replications produced variability indices in the range of 10-12 which can be considered as fairly acceptable.

3.4 Varietal evaluation

A total of 100 rice germ plasm accessions were evaluated on the basis of leaf area fed by the V instar larvae in 48 h of caging. The leaf damage in test varieties (table 5) ranged from 70.8 ± 6.2 mm² (Choorapundy) to 402.9 ± 25.7 mm² (Company Chittari). Statistical comparisons within given set of testing (*t* test) indicated all the varieties with leaf damage less than 200 mm² to generally differ significantly from the susceptible check TN 1.

Consistency in area of leaf damage in susceptible and resistant checks in different sets of evaluation is presented in table 6. Though mean values for each check variety varied slightly among the different sets of evaluations, these values did not differ significantly. Thus, other environmental factors like temperature, humidity, photoperiod etc. could not have drastically affected the feeding by the V instar

Table 5. Performance of selected rice varieties against the leaf folder damage in greenhouse*.

Variety	Leaf area damage (mm ²)
	Mean \pm SE
Choorapundy	70.8 \pm 6.2
Gorsa	94.6 \pm 17.2
Ptb 33	113.8 \pm 22.2
Ptb 19	118.3 \pm 10.2
T 10	125.0 \pm 16.1
ARC 10660	129.4 \pm 11.6
RP 2068-18-4-5	138.6 \pm 25.6
Kula Peruvella	148.2 \pm 16.1
Balam	151.2 \pm 26.7
Ptb 12	160.9 \pm 33.3
Chempan	163.5 \pm 12.3
IET 5742	167.4 \pm 21.6
W 1263	167.5 \pm 12.4
Chemban	170.4 \pm 21.3
Co 29	172.0 \pm 40.1
Darukasail	181.8 \pm 35.6
ARC 7064	187.0 \pm 17.6
MO I	189.6 \pm 25.4
T 1406	191.8 \pm 26.6
IET 5741	268.3 \pm 22.2
ARC 6605	280.3 \pm 28.3
Badhsabhog	310.8 \pm 35.1
ARC 10744	323.2 \pm 26.6
ARC 10840	360.8 \pm 40.9
Company Chittary	402.9 \pm 25.7
TN 1 (susceptible check)	330.0 \pm 26.7

*No. of replications varied from 4 to 10.

Table 6. Leaf area damage recorded in susceptible and resistant varieties by V instar *C. medinalis* larvae in different sets of feeding tests.

Set	Month	Year	Leaf area damage (mm ²) in	
			TN 1 (susceptible check)	PTB 12 (resistant check)
			Mean \pm SE	Mean \pm SE
1.	March	1985	299.8 \pm 14.4 ^a (5)	—
2.	August	1985	341.5 \pm 34.0 (8)	—
3.	April	1986	358.7 \pm 27.6 ^b (9)	207.0 \pm 27.9 ^c (5)
4.	August	1986	330.0 \pm 26.7 (8)	152.0 \pm 29.7 (9)
5.	April	1987	316.8 \pm 30.0 (12)	116.3 \pm 27.1 ^d (9)
6.	May	1987	315.0 \pm 32.9 (7)	170.0 \pm 34.0 (6)
Average			326.9	161.3

Numbers in parentheses are replications.

Comparison of means: Resistant check differed significantly from susceptible check in each set of testing ($P < 0.05$).

a-b, c-d, Not significant (t test).

larvae during the brief period of test. Based on area of leaf damage, either in terms of absolute values or more reliably on relative terms with reference to the

susceptible check variety, the test varieties can be rated for their resistance to the leaf folder.

4. Discussion

Leaf feeding in different larval instars noted in this study indicated highest amount and rate of feeding by V instar larvae. All the larvae observed underwent 5 instars prior to pupation. Though 6 instars were exceptionally observed in this insect by Rajamma and Das (1969) and Vyas *et al* (1981), other workers have reported 5 instars (Lingappa 1972; Velusamy and Subramanian 1974; Wada 1979).

Though leaf feeding gradually increased in amount as larvae developed, daily fluctuations were noted when feeding was monitored continuously (figure 1). Decline in feeding prior to moulting and its subsequent increase may have caused such fluctuations. Thus, the inter-moult period would give higher consistency in feeding rate. In view of the highest feeding rate ($151.1 \text{ mm}^2/\text{day}$) and the longest feeding duration (5.25 ± 0.25 days) V instar larvae were considered most suitable for the feeding test. Larval feeding for 48 h duration was taken as optimal as it allowed sufficient time for larvae to settle down and feed actively while not prolonged enough for attaining prepupal quiescence.

In studies to note factors influencing leaf feeding, V instar larvae recorded no significant differences in area of damage while feeding on 30, 45 and 60 days old TN 1 plants. Nevertheless, there was a trend of decreased feeding on older plants. Younger host plants appeared to be more suitable for this insect as larvae reared on rice leaves at ripening stage showed prolonged development (Wada and Kobayashi 1980) and underwent 6 larval instars (Wada 1979). However, the exposure period in the present study being brief in terms of total larval duration, variations in amount of feeding were not significant. This fact would allow greater flexibility in adopting the feeding test because it would permit 3 to 4 weeks time to carry out the evaluation. Plants younger than 30 days old age presented practical problem in measuring the area of leaf damage. Either V instar larvae totally fed on the leaves without leaving even the epidermis and vascular bundles or damaged leaves succumbed and wilted before measurements could be made.

The feeding test with V instar larvae on TN 1 variety plants raised at two different levels of nitrogen fertilization also did not indicate significant differences in amount of damage. However, leaf nitrogen level in these two batches of plants differed significantly. Many reports are available indicating higher buildup of leaf folder in fields receiving higher levels of nitrogenous fertilizer (see Upadhyaya *et al* 1981). This would imply greater amount of feeding by larvae on plants with greater leaf nitrogen content. Probably, the brief exposure period again might have limited the full expression of such effects in this feeding test.

Besides host plant age and leaf nitrogen content, ambient temperature is most likely to influence feeding by larvae. No separate studies were made at different constant temperatures as the feeding test was contemplated to be used to evaluate a set of varieties at one time and only the relative differences with the check variety are to be considered. Nevertheless, several sets of evaluations conducted during different months of the year with varying maximum and minimum temperatures did show relative consistency with reference to the check varieties (table 6).

Inherent variability among individual V instar larvae in feeding rate was

acceptable with variability index in the range of 10–15 with only 5 replications. Though 10 replications would be ideal, with 5 replications more test varieties can be handled.

It is important to note that the proposed feeding test which was insensitive to plant age and leaf nitrogen level could bring out varietal differences distinctly. Area of leaf damage among test varieties in the feeding test encompassed a wide range. These values showed statistically significant differences among the test and check varieties and were also fairly consistent over a period of 24 months. PTB 12, identified as resistant check in this study also performed well against the pest in field evaluation at different test locations in India under the coordinated programme (DRR 1986). Further, 4 of the varieties viz. Choorapundy, Gorsa, Darukasail and Balam with damage scores 1 and 3 in greenhouse evaluation at IRRI (Heinrichs *et al* 1985) also registered low feeding damage in our test. We thus propose the larval feeding test as an alternative method of greenhouse evaluation of varieties against leaf folder *C. medinalis*.

Based on values obtained in the present screening of varieties, entries could be grouped as those displaying less than 100 mm² damage, 100–150, 150–200 and above 200 mm² damage. Only the latter group of varieties, in general, did not significantly differ from the susceptible check TN 1 in set-wise comparisons. However, we do not propose to use the absolute values of leaf damage in rating varieties as such values are not universally constant. However relative basis of damage can be considered to score varieties. The principle of feeding test proposed can also be adopted with suitable modifications for evaluation of varieties against other leaf scraping rice pests like rice hispa, *Di cladispa armigera* (Oliv.) and caseworm *Nymphula depunctalis* (Guenee).

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Inherited and rearing components of aggressive dominance and autonomic reactivity in the rat

SUNITHA SHANKAR and MEWA SINGH*

Bio-Psychology Laboratory, University of Mysore, Manasagangotri, Mysore 570 006, India

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Abstract. On the basis of their scores in the open field test apparatus and 'limited access' competition, rats were divided into high and low aggressive and high and low reactive lines. Intrastrain breeding experiments were conducted across 3 generations to determine the heritability of aggressive behaviour and reactivity. In each generation, the animals of each strain were reared under 'normal', 'crowded' and 'food restricted' conditions to determine the extent of the influence of rearing environment on these behaviours. The genetic and environmental contributions to aggression and reactivity were further investigated through reciprocal cross matings and foster rearing. In most of the cases, environmental factors showed significant but varied and unpredictable influences, whereas the genetic factors were more consistent in their effects.

Keywords. Aggressive behaviour; autonomic reactivity; inheritance; environmental influences.

1. Introduction

Aggression is a catch-all term and its expression may include components which could be physiological, emotional or cognitive. Behaviour geneticists have shown a considerable interest in the inheritance of various aspects of aggression (Taylor 1980; Broida and Svare 1982; Moss *et al* 1982) and 'high' and 'low' aggressive lines have been established in laboratory animals (Hyde and Sawyer 1980). Further studies involving diallel cross matings have shown the stability of inheritance of aggression on the one hand (Hahn and Huber 1982) and sex-related differential inheritance patterns on the other (Hood and Cairns 1988). On the other hand, experience and learning during development were found to significantly influence aggressive behaviour (Wuensch and Cooper 1981). Two environmental factors, population density (Calhoun 1962; Leyhausen 1965; Aspey 1977) and food availability and distribution (Boccia *et al* 1988) are known to directly affect aggressive behaviour.

Autonomic reactivity or emotionality is 'a complex of factors . . . a group of organic, experiential and expressive reactions . . . general upset or excited condition of the animal' (Hall 1934). A number of studies (DeFries *et al* 1978; Webster *et al* 1979; Marangos *et al* 1987) have shown a strong genetic component in autonomic reactivity which may suggest the possibility of establishing 'high' and 'low' lines through selective breeding. However, developmental conditions such as rearing in small litters (Akuta 1979), crowding (Levitt and Bennet 1975) and handling during infancy (Ader 1968) etc. also influence reactivity to a considerable extent.

*To whom all the correspondence should be addressed.

A relationship has been shown to exist between aggression and emotionality (Yumatov *et al* 1988). In most of the earlier studies, both genetic and environmental factors have been shown to affect aggressive behaviour and reactivity. The present study was designed to assess the relative contribution of genetic and environmental factors on the expression of aggressive behaviour and autonomic reactivity. The terms aggression or aggressive dominance refer to the expression of overt dominance in competition situations involving limited access to a resource. Although 'limited access' technique of measurement of aggression was criticized by Syme *et al* (1974), Pillai and Singh (1984) re-established 'limited access' to be a reliable measure. The technique has also been successfully employed by other investigators (Benton *et al* 1980; Boccia *et al* 1988).

2. Methods

2.1 Subjects, experimental design and procedure

A total of 482 albino rats of the Wistar stock served as subjects.

2.2 Experiment 1: Parental generation

Rats (126) were tested for autonomic reactivity and aggressive dominance in competitive situations. Based on their scores, the animals were divided into 4 groups to establish 4 strains, viz. high aggressive-high reactive (HA-HR), high aggressive-low reactive (HA-LR), low aggressive-high reactive (LA-HR) and low aggressive-low reactive (LA-LR). Males and females were, then, subjected to intrastrain breeding.

2.3 Experiment 2: First generation

The offspring from each strain were distributed into 3 rearing conditions:

Condition A: Normal rearing—one individual per small cage (25 × 10 × 16 cm).

Condition B: Space restricted (crowded)—8 individuals of like sex per large cage (36 × 30 × 22 cm).

Condition C: Food restricted—3 rats of like sex per large cage, provided, on average, 2 g per rat lesser food per day than the amount normally required at any age. The food was provided through an access available to only one rat at a time.

This way, 4 strains, 3 rearing conditions in each strain and sex, formed a 4 × 3 × 2 factorial design (for reactivity). Sex, however, as an independent variable was not considered in aggressivity since males and females were not pitted against each other in competitive situations. At the age of about 80 days, the animals were first tested for autonomic reactivity and immediately after for aggressive dominance. Tests for aggressive dominance were conducted as follows.

2.3a Intrastrain-intercondition tests: Animals of the same strain but from different rearing conditions competed with each other.

2.3b *Intracondition-interstrain tests*: Animals from like rearing conditions but belonging to different strains competed with each other.

After the testing was over, the first generation animals were subjected to strain breeding in order to obtain the second generation stock.

2.4 *Experiment 3: Second generation*

The second generation experiment was a repetition of the first generation experiment.

2.5 *Experiment 4: Reciprocal crosses*

HA-HR and LA-LR animals of experiment 3 were subjected to reciprocal cross mating in the following manner: (i) HA-HR female \times HA-HR male, (ii) LA-LR female \times LA-LR male, (iii) HA-HR female \times LA-LR male and (iv) LA-LR female \times HA-HR male. The offspring, thus consisted of 2 inbred and 2 hybrid groups. The animals in 4 groups were raised in identical manner as 2 animals of like sex per cage.

At the age of 85 days (± 2 days), the animals were tested for autonomic reactivity. For the aggression tests, the animals from 4 different groups competed with each other in pairs and groups of 4, for the 3 competitive tests. Statistical analyses were carried out not only to assess the relative contribution of inbreeding and cross breeding, but also of male and female parentage in cross breeding to the total variance in behaviour.

2.6 *Experiment 5: Foster rearing*

HA-HR and LA-LR animals of experiment 3 were subjected to inbreeding. For the rearing purpose, the obtained offspring were distributed into 4 groups in the following manner: (i) offspring of HA-HR parents—reared by HA-HR mothers, (ii) offspring of LA-LR parents—reared by LA-LR mothers, (iii) offspring of HA-HR parents—reared by LA-LR mothers and (iv) offspring of LA-LR parents—reared by HA-HR mothers. After weaning, the animals were formed into two groups of 'mother-reared' and 2 groups of 'foster reared' subjects.

2.7 *Methods for assessment of autonomic reactivity and aggressive dominance*

2.7a *Autonomic reactivity*: The open field test apparatus was used to measure autonomic reactivity. The animal was exposed to light and sound stressors for 2 min each day for 5 days. Autonomic reactivity was determined by taking the mean defecation score obtained through the 5-day test.

2.7b *Aggressive dominance*: Measures of aggressive dominance were obtained in tests involving competition for food or water. Three tests, each after 48 h of deprivation, were conducted as follows:

- (i) Pair-wise competition for food (15 min).

- (ii) Group competition (4 animals at a time) for food (15 min).
- (iii) Group competition for water (15 min).

Two-day regular feeding schedule was maintained between the tests.

The competition was for the source (food or water) which was accessible to only one animal at a time. Although each rat was involved in all the 3 tests, no animal was made to compete with another animal more than once in order to avoid the influence of any possible dominance hierarchy established during the previous competition.

During the experiment on parental generation, the variables recorded were body-weight before and after deprivation, body weight after the competition, time spent at the source during the contest and successful and unsuccessful attempts at displacing the competing rats from the source. These data were subjected to repeated intercorrelations, and it was established that the 'weight gain' and 'time spent' at the source during the contest were the most reliable measures of aggressive dominance. The details of these calculations are reported elsewhere (Pillai and Singh 1984). A single aggressivity score for an animal was calculated as follows:

$$(i) c = \frac{\text{Total time spent at the source by all animals}}{\text{Total gain in body weight of all animals}}$$

$$(ii) \text{ Aggressive dominance score} = \text{gain in body weight} + \frac{\text{Time spent at the source}}{c}$$

for a single animal.

c is a constant which indicates amount of food ingested per unit time by any animal irrespective of level of aggressivity.

2.8 Apparatus

2.8a Open field test apparatus: The apparatus consisted of a circular arena, 82 cm in diameter and 32 cm in height, made of hard cardboard. The white floor was marked into segments of equal area in a series of 3 concentric circles. The arena was placed in a 60 cm high, semi-sound insulated box. A 0.7 cm thick transparent glass covered the box. The stress stimuli were 8 bulbs of 100 watts each and high pitch sound generated on an audio-oscillator and fed to a loud speaker via an amplifier. A time switch controlled the duration of light and sound.

2.8b Limited access test cage: The test cage had dimensions of 36 × 30 × 22 cm and was made of thick wire mesh, which facilitated direct observations from all sides and top. The access which was situated at the back of the cage measured 4 × 4 cm and allowed only a single rat to the resource at a time. The access was kept shut with a sliding plate which was removed only at the onset of the experiment.

3. Results

3.1 Experiment 2: First generation

3.1a Autonomic reactivity: The obtained F values were significant for strain ($F =$

6.07; $P < 0.01$), reactivity ($F = 14.74$; $P < 0.01$), rearing ($F = 3.73$; $P < 0.05$), sex ($F = 9.06$; $P < 0.01$) and strain and sex interaction ($F = 3.34$; $P < 0.05$). HA-HR and LA-HR strains (means 2.49 and 2.61 respectively) scored significantly higher on reactivity than LA-LR strain (mean 1.38). Irrespective of the aggressivity background, the descendents of HR parents scored significantly higher than the descendents of LR parents (means 2.55 and 1.67 respectively). With regard to the effect of rearing conditions, the crowded and the food restricted rats (mean 2.21 and 2.43 respectively) were more reactive than the rats reared under normal condition (mean 1.69). A significant strain \times sex interaction revealed that whereas among males HA-HR strain scored highest on reactivity, the same happened in LA-HR strain among the females.

3.1b Aggressive dominance: Intrastrain-intercondition tests: The analysis indicated a highly significant effect of rearing conditions ($F = 116.39$; $P < 0.01$) and rearing \times sex interaction ($F = 10.78$; $P < 0.01$) on aggressive dominance scores. The scores on aggressive dominance by crowded rats (mean 16.38) and food restricted rats (mean 15.47) were significantly higher than those of normally reared rats (mean 9.21). A significant rearing \times sex interaction revealed that the effect was stronger on males than on females (mean scores: females; 8.64—normal, 13.90—crowded, 12.72—food restricted; males; 9.79—normal, 18.87—crowded, 18.22—food restricted).

3.1c Intracondition-interstrain tests: The analysis indicated that 4 major strains as well as 'aggressivity' as a strain differed significantly amongst each other ($F = 3.87$; $P < 0.05$ and $F = 10.27$; $P < 0.01$ respectively). The mean score (15.35) of 'HA' (descendents of HA parents) animals was significantly higher than the mean score of 13.71 of 'LA' (descendants of LA parents) animals. The 't' tests indicated that HA-HR and HA-LR strains had higher aggressivity scores (means 15.61 and 15.10 respectively) than LA-HR (mean 13.34) and LA-LR (mean 14.05) strains.

3.2 Experiment 3: Second generation

The second generation experiment was a repetition of the first generation experiment to re-confirm the findings.

3.2a Autonomic reactivity: As far as the major strains and 'reactivity' as a strain were concerned, the results in the second generation experiment were the same as in first generation. The mean reactivity score of HR animals (3.07) was significantly higher than the mean score of LR animals (mean 1.86), indicating inheritance of autonomic reactivity. Rearing conditions, however, failed to show any significant effect on reactivity.

3.2b Aggressive dominance: The analysis of variance for the data obtained through intrastrain-intercondition and interstrain-intracondition tests revealed the findings to be identical to those of experiment 2—first generation experiment, of course with different individual and mean values.

3.3 Experiment 4: Reciprocal crosses

3.3a *Autonomic reactivity*: The analysis of variance computed for autonomic reactivity revealed no differences among the mean scores of the 4 groups ($F=0.664$).

3.3b *Aggressive dominance*: The analysis revealed that the mean difference among the strains for aggressive dominance was statistically significant at 0.05 level ($F=3.92$). A further analysis indicated that the offspring of both HA parents scored higher on aggressive dominance (mean 14.24) than the other 3 strains (HA-HR \times LA-LR = 10.04; LA-LR \times HA-HR = 9.61; LA-LR \times LA-LR = 9.44).

3.4 Experiment 5: Foster rearing

3.4a *Autonomic reactivity*: The analysis revealed significant differences between the HR and LR strains (means 3.09 and 1.72 respectively), between mother and foster rearing (means 2.01 and 2.80 respectively) and among the 4 groups ($F=6.33$; $P<0.01$). The HR strain animals reared by their biological mothers obtained higher scores (mean 2.88) than the LR strain animals reared by their own mothers (mean 1.13). The foster reared HR strain (mean 3.33) scored higher than the mother reared LR strain, and the LR strain foster reared by HR females (mean 2.30) obtained higher scores than the LR mother reared strain. Foster rearing enhanced the reactivity scores in both the strains, but the effect was more striking in the case of LR animals reared by HR females than in the HR animals foster reared by LR females.

3.4b *Aggressive dominance*: The analysis indicated the F values to be significant for strains ($F=4.32$; $P<0.05$), groups ($F=8.03$; $P<0.01$), strain \times rearing interaction ($F=16.90$; $P<0.01$) and strain \times rearing \times sex interaction ($F=5.57$; $P<0.05$). The HA strain animals reared by their own mothers (group I, mean 13.13) scored significantly higher than LA animals reared by their own mothers (group III, mean 7.61). HA animals reared by LA females (group II, mean 10.98) also scored higher than group III. LA animals reared by HA females (group IV, mean 12.79) scored significantly higher than group III animals of the same strain. The significance of the strain \times rearing interaction can be seen by comparing the means of group I with group II and of group III with group IV. Whereas in the HA strain, foster rearing by LA females reduced the aggressivity, the difference between the 2 groups was not significant. On the other hand in the LA strain, the foster rearing by HA females significantly increased the aggressivity. The 3-factor level significant interaction for strain \times rearing \times sex was evident from the means of various groups. The effect of foster rearing was absent in HA strain in both sexes. Furthermore, in the LA strain the foster rearing significantly increased aggressivity only in the case of males (means 6.09 and 14.52, $t=3.60$), whereas the effect in the case of females was statistically non significant.

4. Discussion

4.1 *Autonomic reactivity*

The animals in the parental generation were divided into HR and LR groups on the basis of their scores in the open field test apparatus. In both first and second

generation experiments, the offspring born to HR parents scored significantly higher on reactivity than the offspring born to LR parents. As far as inheritance of reactivity was concerned, the genetic determination showed up clearly, irrespective of the aggressivity of the animals in the 4 strains. Emotionality, being reactivity of autonomic nervous system, though modifiable to some extent by environmental factors, is more or less a physiological trait. The genetic determination of such behaviour traits that have strong physiological correlation is very much expected.

In the first generation experiment, crowding and food restriction significantly enhanced the reactivity when compared with normal rearing, irrespective of the genetic background. This finding also corresponds to the earlier studies mentioned in the 'introduction' regarding the nature of environmental factors influencing reactivity. The added feature of the present study was a repetition of the experiment using the same design. Surprisingly, in the second generation, the same environmental conditions failed to affect the reactivity of the animal. The results indicate that environmental influences are varied and unpredictable, and unlike inheritance of such traits, generalizations cannot be made even for as severe conditions as the ones employed in the present study.

The outcome in the reciprocal cross experiment was extremely interesting. Although the inbred HR and LR groups had significantly higher and lower scores respectively than the two cross bred strains, the differences were not statistically significant. These results add two more observations to the genetic aspect of autonomic reactivity: (i) mating between one HR and one LR parent produces offspring with reactivity scores in between the two parents, indicating the trait to be polygenic and its mode of inheritance to be 'intermediate', and (ii) there is no differential effect of male or female parental genes on the autonomic reactivity of the offspring.

The experiment on foster rearing also indicated interesting patterns about the role of rearing by the females. Although the presence of true breeding of the trait was according to the genetic hypothesis, the effect of foster rearing was not as one would expect it to be. According to the hypothesis of environmental influences, one would expect a decrease in reactivity of animals foster reared by LR females, and vice versa. However what was observed in the present study was that foster rearing, of both types, enhanced the reactivity scores in both strains, so much so that the reactivity scores of foster reared animals were higher than those of mother reared animals irrespective of strain. The results once again indicated that the environmental influences, unlike genetic effects, do not follow any specified or predictable pattern.

4.2 *Aggressive dominance*

Although the animals in the first generation experiment were divided into 4 strains, they could also be considered belonging to two groups viz. HA and LA, ignoring their scores on autonomic reactivity. Since the offspring of HA strain scored significantly higher on aggressive dominance than the offspring of LA strain, the results revealed the true breeding nature of the trait.

In addition to the significance of difference between HA and LA strains, two more observations in this experiment were worth noting: (i) except strains (of both types), no other differences were found to be significant, and (ii) when the

competition tests conducted were among the animals from identical rearing conditions of different strains, the rearing variable showed a non-significant contribution to the total variance.

When the experiment was repeated, the second generation animal showed the same pattern of aggressivity scores as their parents in the first generation. Although the inheritance pattern came out more clearly in the second generation experiment, a few significant contributions, not observed in the first generation, were also recorded. One such observation was a significant contribution of strain \times sex interaction. Although when combined, the HA strain animals scored higher on aggressivity than the LA strain, the difference was more marked in the case of males than females. Looking at the overall results of the two generations, one fact that remarkably stands out is the consistency of inheritance of aggressivity in the strains.

In the first generation experiment, the rearing factor showed a highly significant effect on aggressive dominance. The crowded and the food restricted animals were found to be more aggressive than the normally reared animals. The significance of rearing \times sex indicated that the effect of environmental conditions was more marked in the case of males than females. The second generation experiment brought out exactly the same results. A very important aspect of environmental influences is the severity of environmental conditions. The manipulated rearing conditions in the present study were so severe that their influence on aggression is not surprising at all. The food restricted animals not only received lesser food on the average, but also had to compete with each other through the limited access throughout their development. These animals can be expected to show higher aggression than the crowded animals who never had food shortage. Such a difference, however, was not observed. Since the average space available to each crowded animal was half of that for the food restricted animal, sensitivity to 'absolute space' could have been the factor enhancing aggressivity in crowded animals. It can be hypothesized that the environmental component of variance may keep on changing, from small to very high, depending upon how mild or strong are the manipulated variables, but the genetic component may always show a consistency.

The experiment involving reciprocal crosses brought out results, as would be expected according to the genetic hypothesis, about a trait which is probably polygenic. The average score on aggressive dominance tests was lowest in the LA strain and highest in the HA strain animals. The scores of the animals produced by reciprocal crosses ranged between the high scoring and the low scoring parents. When all 4 groups were compared, the difference was found to be significant only between the offspring of HA parents and the other 3 groups, and not amongst the 3 groups themselves. The findings suggest that the genes influencing the trait of aggressivity do not seem to indicate any differential inheritance related to sex.

The effect of foster rearing on aggressivity was rather complicated and diverse. The HA strain scored higher than the LA strain. When the mother reared animals (irrespective of strain) were compared with foster reared animals, the obtained *F* value was non-significant, indicating no effect of fostering. A further analysis, however, revealed the case not to be so. The non-significance of the difference between means was because foster rearing of HA strain by LA females reduced their aggressivity only non-significantly but foster rearing of LA strain by HA females enhanced their aggressivity significantly.

Rearing \times sex interaction indicated that the enhancement of aggressivity in foster reared LA strain was significant in the case of males only. It was also observed that crowding and food restriction enhanced aggression significantly only in males. This kind of differential environmental effect on the sexes has enormous significance for the understanding of evolution of behaviour patterns in the two sexes. In natural environment, aggressive behaviour can be beneficial to the males (holding territories or organized into linear dominance systems) but harmful to females. It is possible that there is natural selection for some genes in males to respond more quickly and strongly to environmental pressures than females. In fact, Haug and Mandel (1978) found in mice that the aggression of the two sexes was under separate genetic or hormonal control. Singh *et al* (1984) reported that even in monkeys, the behavioural responses to harsh and hostile environments are more marked in males, even to the extent of resulting in a higher male mortality as compared to females.

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Effect of imipramine and amitriptyline on circadian locomotor activity rhythm in the field mouse *Mus booduga*

P SUBRAMANIAN and R SUBBARAJ*

Department of Animal Behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. We have performed experiments on the influence of imipramine and amitriptyline (antidepressant drugs) offered through drinking water on the period length (τ) of the circadian locomotor activity rhythm in the field mouse *Mus booduga* under continuous darkness (DD). Ingestion of imipramine to mice under freerunning conditions caused lengthening of the activity rhythm whereas amitriptyline caused dissociation of activity components. Furthermore, the amount of activity (α) is significantly reduced ($P < 0.005$) during imipramine as well as amitriptyline treatment. The action of these antidepressants may be explained by their action on serotonergic terminals in the suprachiasmatic nucleus, the putative circadian pacemaker in mammals.

Keywords. *Mus booduga*; period lengthening; imipramine; amitriptyline; amount of activity.

1. Introduction

Drugs have proven to be valuable in probing the mechanism(s) underlying mammalian circadian system (Wehr and Wirz-Justice 1982). Several investigators have described the period modifying effects of antidepressant drugs on circadian rhythms (Wirz-Justice and Campbell 1982). Lithium ions were first reported to cause period lengthening in the circadian rhythm of petal movement of a crassulacean flower and in the locomotor activity of a desert mouse (Engelmann 1973) and has since been known to have significant effects in several other systems (Subbaraj 1981). Imipramine, one of the tricyclic antidepressant drugs was reported to cause period lengthening and dissociation of activity components in the locomotor activity of freerunning hamsters kept under constant darkness (DD) (Wirz-Justice and Campbell 1982). Clorgyline, another type of antidepressant drug, was also reported to promote dissociation of activity components (Duncan *et al* 1988). Furthermore, clorgyline and imipramine were found to delay the phase position of many neurotransmitter receptor rhythms (Wirz-Justice *et al* 1982).

We have studied the influence of imipramine and amitriptyline (tricyclic antidepressant drugs) on the locomotor activity rhythm of the field mouse *Mus booduga* offered through drinking water. Imipramine was found to cause period lengthening of the activity rhythm under freerunning conditions in DD whereas amitriptyline dispersed the activity components of the freerunning rhythm. There was a significant reduction of amount of activity (α) during the treatment of both these drugs.

*To whom all the correspondence should be addressed.

2. Materials and methods

The mice were captured from fields surrounding the University campus. They were kept in light-tight, temperature controlled experimental cubicle maintained at $31 \pm 1^\circ\text{C}$. All experiments were performed in constant darkness (DD). Food comprising of millets, maize, grains and water were available *ad libitum*. Dim red light of 610–700 nm was used while feeding the animals (Viswanathan and Chandrashekar 1985) and the hours of routine care were varied. An eccentrically placed magnet on the wheel temporarily made and broke contacts in an electrical circuit with every turn of the wheel. The revolutions were picked up by channels of an A 620X Esterline Angus Event Recorder. Actograms were constructed and double plotted in a manner which is now routine in chronobiological research (Pittendrigh and Daan 1976).

Imipramine (2 mg/ml) and amitriptyline (2 mg/ml) were administered through drinking water for its wide acceptance (Subbaraj 1981; Aschoff 1989) and as a reliable and convenient mode of drug administration (Melzack *et al* 1985). (LD_{50} for imipramine = 3.5 mg/ml; LD_{50} for amitriptyline = 3 mg/ml for *M. booduga*). Stable values of period length (τ) were established while mice were in DD and drinking tap water. After 14 days of freerun, the water bottles were replaced with particular concentration of the drug. Drug administration was continued for the consecutive days of the experiment. For pharmacological studies on circadian rhythms, each animal acts as its own control (Kayser and Hildwin 1977). Final steady values of period length (τ) obtained while mice were ingesting the drug were calculated and compared to τ prior to drug administration.

3. Results

The activity recordings of imipramine treated animals are shown in figure 1. Imipramine had period lengthening effect in all the cases studied ($n=7$). Period change was observed immediately after the administration of the drug. The values of τ (mean \pm SD) were 23.50 ± 0.25 h before treatment and 23.62 ± 0.25 h after imipramine treatment ($P < 0.05$; Student's *t*-test). Dissociation of activity components of the freerunning rhythm was observed during the treatment of amitriptyline (figure 2) ($n=6$). The amount of activity (α) became lesser during the treatment of both these drugs. The reduction of α during drug treatment is statistically significant ($P < 0.005$, Student's *t*-test; table 1). After the termination of drug treatment, an increase in the amount of activity was observed.

4. Discussion

The mice *M. booduga* exhibit very precise onset on nocturnal motor activity which permits an accurate measurement of τ . Furthermore, the mice exhibit very stable period length (τ) and the exogenous influences have very little effect. Thus, this resistance to manipulation lowers the probability of spurious results (Viswanathan and Chandrashekar 1985).

Antidepressant drugs are reported to lengthen τ , dissociate activity components and to reduce amount of activity (Wirz-Justice and Campbell 1982; Aschoff 1989) in

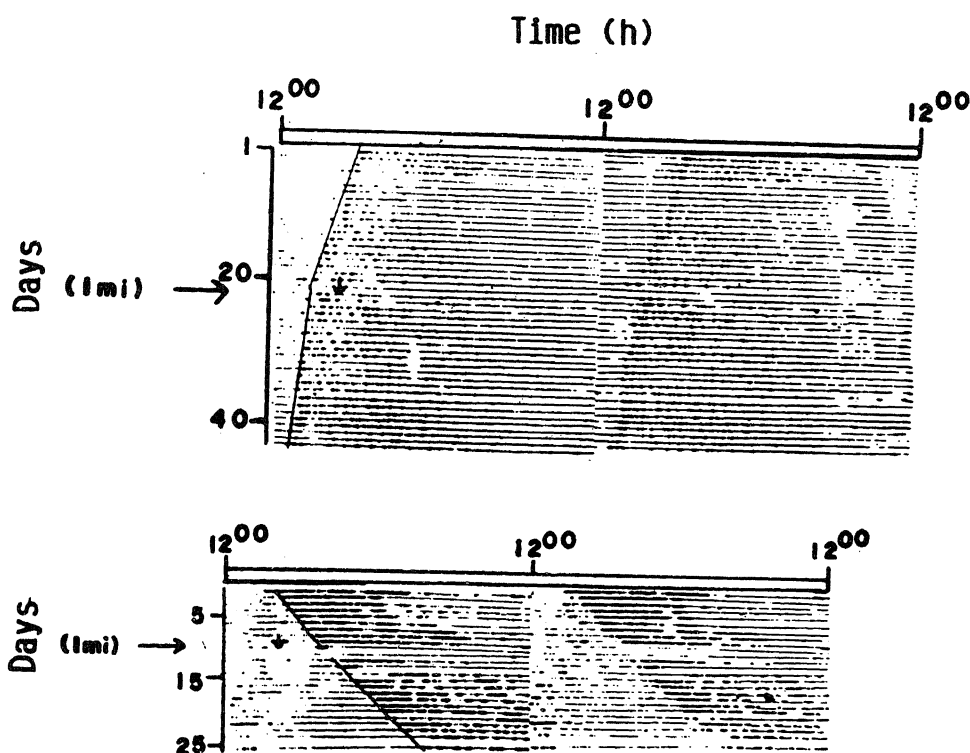


Figure 1. Representative actograms showing the effect of imipramine (2 mg/ml) on freerunning rhythm of *M. booduga*. A period lengthening occurred after the drug treatment.

hamsters and our studies substantiate these findings. Moreover, the magnitude of change in τ during drug treatment is small and a difference of only 0.2–0.3 h has been documented (Wirz-Justice and Campbell 1982). In our studies also, similar results were obtained. Antidepressant drugs are known to exert their mechanism by increasing serotonin levels in suprachiasmatic nucleus (SCN), the putative circadian hypothalamic pacemaker (Greco *et al* 1988). The SCN is particularly rich in serotonergic terminals (Groos *et al* 1983) and it was proved that imipramine binding in the SCN was highest than in any other brain regions (Wirz-Justice *et al* 1983). Such pharmacological manipulation of serotonin might modify circadian phase or period in mice. For instance, Wirz-Justice *et al* (1982) reported that clorgyline delayed the phase position of several neurotransmitter receptor rhythms in rats under entrained conditions and suggested that these delayed phase position of receptors may be reflected in the frequency of the central circadian pacemaker.

Dissociation of activity components was observed during the treatment of amitriptyline in mice similar to the treatment of clorgyline in hamsters (Duncan *et al* 1988). The period lengthening effect of imipramine and dissociation of activity components of amitriptyline may also be explained by the predictions based on the Pittendrigh-Daan model of the complex circadian pacemaker (Pittendrigh and Daan 1976). According to this model, the phase relationship between two coupled

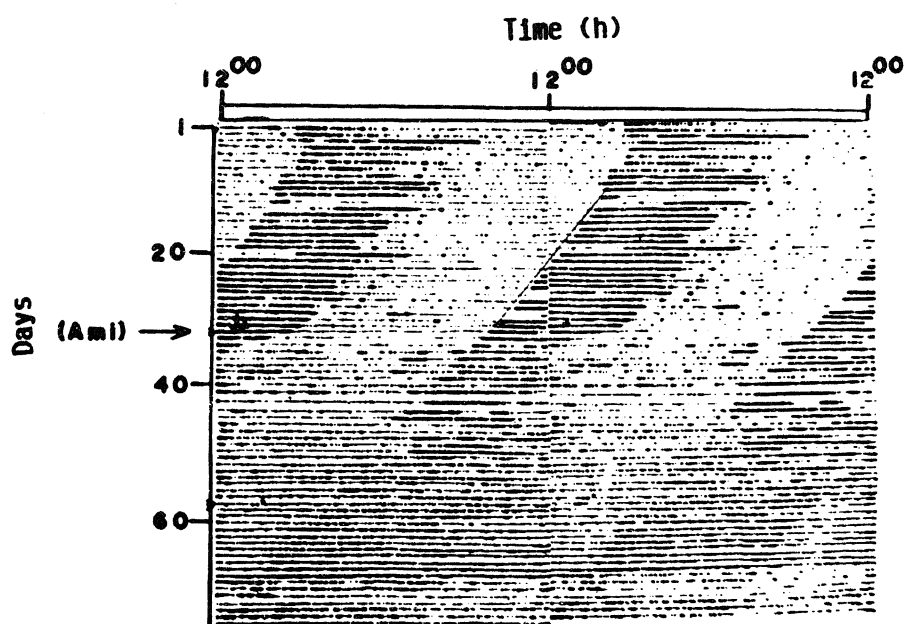


Figure 2. Effect of amitriptyline (2 mg/ml) on the freerunning rhythm of *M. booduga*. Dispersed activity components can be seen after the drug treatment.

Table 1. Effect of imipramine and amitriptyline on the amount of activity.

Amount of activity (α)					
Before drug treatment (h)		During drug treatment (h)		After drug treatment (h)	
Imipramine (2 mg/ml)	Amitriptyline (2 mg/ml)	Imipramine (2 mg/ml)	Amitriptyline (2 mg/ml)	Imipramine (2 mg/ml)	Amitriptyline (2 mg/ml)
6.91	9.06	4.46	6.96	6.7	10.1
6.7	12.23	4.14	2.13	6.97	4.34
6.97	9.4	6.24	7.48	7.47	7.56
9.66	6.52	6.43	1.28	6.6	3.2
6.6	7.14	6.58	6.54	6.95	6.91
6.9	4.73	3.14	1.47	4.93	1.6

oscillators determines the period of freerunning activity as well as the ratio of activity to rest. Our drug induced changes in the overt locomotor rhythm can be interpreted as resulting from a change in this phase relationship (Duncan *et al* 1988). There was a reduction of α during methamphetamine treatment in hamsters (Aschoff 1989) similar to our results obtained in *M. booduga*. Antidepressant drugs thus provide powerful tools to investigate circadian physiology and its neurochemical basis. Conversely, modulation of circadian frequency may be an important mode of action of widely disparate group of antidepressant drugs.

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Mineralization of phosphorus by faecal phosphatases of some earthworms of Indian tropics

R V KRISHNAMOORTHY

Department of Zoology, University of Agricultural Sciences, GKVK, Bangalore 560 065, India

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Abstract. The casts of earthworms contain greater amounts of extractable inorganic phosphate than underlying soils. Casts contain higher phosphatase activity than that occurs in undigested soil, which increases the inorganic phosphorus released by mineralization. Of the 4 species compared, *Perionyx excavatus* showed a higher faecal phosphatase activity. Composition of casts vary with species. pH increases and nutrients accumulate in worm-activated soils. Based on the data on surface cast production, it is estimated that grasslands around Bangalore show a phosphorus turnover of about $55 \text{ kg ha}^{-1} \text{ y}^{-1}$, and the woodlands, $38 \text{ kg ha}^{-1} \text{ y}^{-1}$.

Keywords. Faecal phosphatase activity; earthworm cast composition; phosphorus turnover in soils; phosphorus mineralization.

1. Introduction

It is now established that alkaline phosphatases are excreted in earthworm faeces as wormcasts (Satchell and Martin 1984; Satchell *et al* 1984). The wormcasts are not only richer in soluble inorganic phosphates but also in exchangeable phosphorus (Sharpley and Syers 1976; Mansell *et al* 1981), indicating the occurrence of active dephosphorylations of phosphorylated compounds in the gut of the worm. The mineralization and mobilization of phosphorus in soils are very much needed especially when farm yard manures are used in agriculture, as they contain most of the P in immobilizable organic form (McAuliffe and Peech 1949). Earthworms' role in P mobilization may thus be relevant and manifest in the disposal of organic wastes as their casts containing phosphatases may help dephosphorylating the immobilizable phosphorylated organic complexes in the soils. A higher phosphatase activity in undigested soil has been demonstrated in the wormcasts of some temperate worms (Satchell and Martin 1984; Satchell *et al* 1984). However, such studies have not been extended to tropical situations. The present paper describes to what extent the earthworms around Bangalore would contribute to the P mineralization in soils.

2. Materials and Methods

2.1 Maintenance of worms and culture

Cultures of 4 species of earthworms, viz. *Lampito mauritii* Kinberg, *Perionyx excavatus* E Perrier, *Pontoscolex corethrurus* and *Pheretima elongata* E Perrier were kept at room temperature in boxes ($10 \times 16 \times 6 \text{ cm}$ each) containing garden soil amended (pH 7.45, 20% moisture) with cow dung from a manure heap in 9:1

proportion. Ten adult (clitellates) worms of each species were introduced in each box and covered with a black cloth to reduce the effects of light on surface active worms. Wormcasts from the cultures were collected for comparison with control cultures of the same soil amendment maintained under the same conditions but without worms. Freshly deposited, unfrozen, pooled casts from 6 to 7 boxes and soil materials were used for analyses.

2.2 *Cultures on paper waste*

Paper waste sludge prepared in the laboratory by soaking and beating blotting paper strips was found to be palatable and habitable to worms. It formed a phosphate-free culture medium. The paper waste paste containing approximately 86% water, was sterilized to minimise microbial growth. Forty g aliquots of this paste was placed in a small polythene box (8 × 10 × 3 cm) containing 20 adult worms or no worms (control). One g of phytin (calcium inositol hexaphosphate) to each 40 g of paper waste was added. The medium can be regarded as simulating the worms' natural diet in so far as paper waste contains mainly of water, cellulose and minerals (ash). The paper waste culture was maintained at desired temperature in a BOD incubator for desired period of time.

2.3 *Phosphatase activity*

Samples of fresh wormcasts or paper wastecasts and of the control media equivalent to 0.5 g dw were collected for phosphatase activity. They were incubated in 20 ml universal buffer solution (BDH) adjusted to the desired pH in a waterbath at 26°C or at desired temperature for 30 min to extract the enzyme. Then it was filtered through a muslin cloth (sterilized and folded 4 times) in a Buchner funnel. Phosphatase activity in the filtrate was determined by the disodium phenylphosphate method (Hoffman 1968) using dilutions of phenol for the standard curve calibration. The phosphatase activity was estimated as µg phenol liberated in 3 h per unit dw wormcast. The enzyme activity was assayed with the buffer providing a pH range of 2-10. The assays were repeated to obtain concurrent results.

2.4 *Development of faecal phosphatase activity*

The paper waste-phytin (2.5%) cultures of *P. excavatus* in 6 boxes were maintained in a BOD incubator. At weekly intervals, one box was removed and the faecal phosphatase in the residue was extracted into the buffer solution pH 8.5, and the activity was measured at the same incubation temperature. The enzyme in the residues obtained at 20, 25, 28 and 30°C were assayed at respective temperatures. Residues without the worms, assayed at 30°C served as controls.

2.5 *Chemical analyses*

The total organic carbon content of soil and casts was determined by the formula $1.8 C\% = 100\% - \% \text{ash}$ (Allen *et al* 1974). The total nitrogen content was

determined by Kjeldahl procedure on samples of about 0.5 g (Jackson 1967). The total Ca, Mg, K and Na were estimated after digesting the material with (2 ml/g) concentrated HCl. The digested material was filtered and assayed. The exchangeable (ionic) Ca, Mg and K were extracted with double distilled water, and filtered through a Whatman No. 1 paper. The filtrate was approximately diluted for determination of the cations by atomic absorption spectrophotometry with LaCl_3 to overcome PO_4 interference. The $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ of soil and casts were extracted with 1 M KCl at a solution: solid ratio 20:1, filtered through a Whatman No. 1 paper and estimated according to Strickland and Parsons (1968). The Cl^- contents of the water extracts were determined by AgNO_3 titration (USEPA 1979). The total phosphorus was extracted with Olsen's extractant (0.5 M NaHCO_3 buffered at pH 8.5), and the inorganic P, with distilled water and estimated colorimetrically by ascorbic acid-molybdic acid method (USEPA 1979). The difference between the total P and inorganic P values was considered to give the organic P content. Dry weights of samples were obtained as loss in weight at 105°C overnight. Ashing of samples was done in a muffle furnace set at 450°C for 20 h. Loss on ignition values were calculated on determining the ash content. All results were calculated and expressed on dry weight basis.

3. Results

The composition of wormcasts varied with species (table 1). The wormcasts were rich in certain components when compared to wormless control soils. The casts of *P. excavatus* were rich in total nitrogen, ammonia nitrogen and nitrate and phosphate contents; whereas the casts of *L. mauritii* were rich in urea content. The

Table 1. Composition of casts of some earthworm species.

Percentage composition	Control soil	<i>L. mauritii</i>	<i>P. elongata</i>	<i>P. excavatus</i>	<i>P. corethrurus</i>
pH	7.45	8.10	8.15	8.25	8.05
Organic C	0.612	0.292	0.298	0.276	0.279
Total nitrogen content	0.091	0.193	0.189	0.208	0.174
C/N ratio	6.72	1.51	1.57	1.32	1.60
Total Ca	0.81	1.39	1.28	1.36	2.01
Total Mg	0.32	0.29	0.31	0.30	0.28
Total K	0.516	0.49	0.52	0.48	0.49
Total P	0.067	0.083	0.091	0.162	0.093
Total Na	0.113	0.120	0.115	0.117	0.121
Exchangeable Ca	0.126	0.184	0.172	0.209	0.398
Exchangeable Mg	0.153	0.231	0.284	0.279	0.261
Exchangeable K	0.099	0.386	0.391	0.381	0.396
$\text{NH}_4^+\text{-N}$	0.002	0.012	0.018	0.092	0.026
$\text{NO}_3^-\text{-N}$	0.0005	0.006	0.0058	0.0092	0.0064
Cl^-	0.73	0.729	0.719	0.730	0.726
PO_4^-	0.0469	0.0699	0.0678	0.1397	0.0711
Urea	0.072	0.155	0.138	0.098	0.089
Total organic P (OP)	0.02	0.0128	0.0227	0.0219	0.021
OP/Pi ratio	0.42	0.18	0.33	0.16	0.28

Values are dry weight percentages.

casts of *P. elongata* were rich in organic phosphorus content. They were more alkaline than the surrounding soil. They were poorer in organic carbon and richer in total nitrogen contents. The C/N ratios were lower in the wormcasts than in the soil. It was also noticed that wormcasts contained more total Ca and P contents but the total Mg, K and Na contents remained more or less unchanged during casting. Total exchangeable cation (like $\text{NH}_4^+\text{-N}$, Ca, Mg and K) concentrations increased in the casts, suggesting that the soil organic matter-bound ions are freed during the passage of soil through the gut. The exchangeable anion concentrations like those of $\text{NO}_3^-\text{-N}$, and PO_4^- were greater in the casts, whereas that of Cl^- remained unaffected. Furthermore, it was found that casts were rich in urea content. The total organic phosphorus content was not changed. Consequently, the organic P/inorganic P ratio reduced in the casts. The extent of these changes obviously (table 1) varied with species. It is evident from the data of table 1 that among the 4 species compared, the casts of *P. excavatus* showed a discrete but the highest degree of these variations.

The effects of casting activity on phosphorus mineralization by the 4 species selected for the study were compared (table 2). The casts of paper waste sludge of two species viz. *L. mauritii* and *P. excavatus* contained more of water-soluble P and mineral contents. More of total P and Pi were extractable from these casts. The reduction on loss-on-ignition values in the casts, showed the decomposition of organic matter present in soils during passage of the soil in the worm's gut. The increases in total extractable P over the control can perhaps be attributed to increased concentration of the mineral matter. However, in the culture medium of *P. excavatus* an increase of 26.15% in the total extractable phosphorus existed which was somewhat greater than that occurring in other cultures of other species. This observation indicates the occurrence of a higher level of mineralization in the worm.

Paper waste sludge maintained for 6 weeks was eaten and casted out by worms.

Table 2. Effects of earthworm faecal phosphatase activity on phosphorus mineralization in paper waste sludge maintained for 6 weeks and difference from controls (% in parenthesis).

Fraction	$\mu\text{g P g}^{-1}$ culture residue dw/20 worms/6 weeks				
	Control	<i>L. mauritii</i>	<i>P. elongata</i>	<i>P. excavatus</i>	<i>P. corethrurus</i>
Water-soluble P	15 \pm 1.56†	25 \pm 1.92* (+ 66.66)	16 \pm 1.38 ^{NS} (+ 6.6)	27 \pm 2.08* (+ 80.0)	17 \pm 1.28 ^{NS} (+ 13.33)
Total extractable P	260 \pm 9.81	318 \pm 9.81* (+ 22.30)	271 \pm 3.87 ^{NS} (+ 4.23)	328 \pm 12.36* (+ 26.15)	279 \pm 6.84 ^{NS} (+ 7.30)
Extractable inorganic P	182 \pm 11.37	268 \pm 14.81* (+ 47.25)	202 \pm 9.67 ^{NS} (+ 10.98)	283 \pm 13.79* (+ 55.49)	212 \pm 8.35* (+ 16.48)
Loss on ignition (%)	48 \pm 2.66	38 \pm 1.93* (- 20.83)	32 \pm 1.69* (- 33.33)	41 \pm 1.26* (- 14.58)	38 \pm 2.03* (- 20.83)
Mineral content** (%)	45 \pm 1.92	51 \pm 2.37* (+ 13.33)	53 \pm 1.62* (+ 17.77)	51 \pm 2.89* (+ 15.55)	51 \pm 3.16 ^{NS} (+ 13.33)

†Mean \pm SD of 7 sets of experimental observations.

*Difference significant ($P < 0.01$).

**Ash content.

NS, Not significant ($P > 0.01$) with reference to controls by Students mean difference 't' test.

The castings of *L. mauritii* and *P. excavatus* when compared to controls were found to be rich in water-soluble P, total extractable P and extractable inorganic P (table 2). Loss of ignition values were lowered in the casts of almost all species compared but the latter had more mineral content (table 2). These results show that mineralization of phosphorus is more in the casts of at least two species than in raw wormless paper sludge. The paper waste contained phytin which was mineralized both by intestinal as well as faecal phosphatases when the former passes through the gut. Mineralization is more in cultures with *P. excavatus* than in cultures with other 3 species (table 2). The faecal phosphatase activity in different species varied but all of them exhibited two peaks of activity with reference to the pH of the incubating medium. The wormless (unbeaten) raw food exhibited only one peak between pH 3 and 4 (figure 1). The other pH activity peak was smaller than this peak and had fallen around pH 9 in the casts of almost all species. It is believed that the peak between pH 3 and 4 might be due to microbial phosphatases (Satchell and Martin 1984) and it is present in the raw food without worms. The other peak found at pH 9 may be due to the phosphatase released by worms' gut (figure 1). The activities of both the phosphatases were higher in the casts of *P. excavatus* than in the casts of other 3 species (table 3). These observations suggested that *P. excavatus* has greater potentialities to mineralize the organic matter. Mineralization by *P. excavatus* in garden soil cultures was found to be greater (table 4) than that in paper sludge cultures with phytin. Increase in mineral residue is also greater in

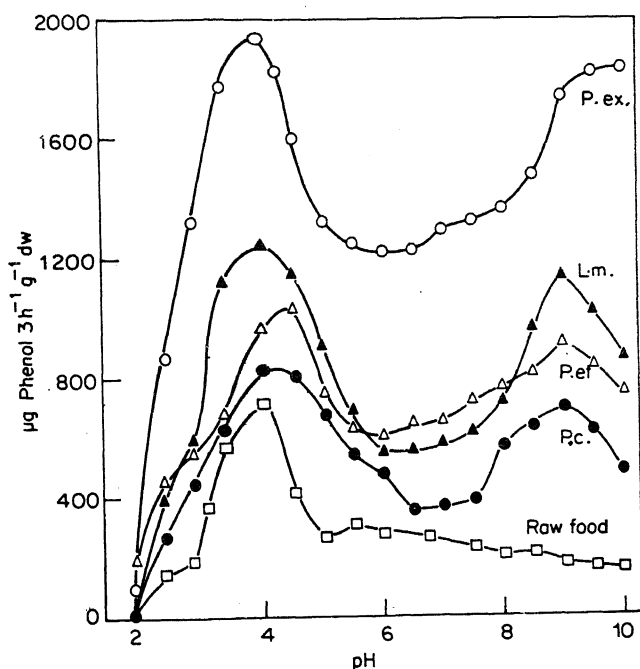


Figure 1. Phosphatase activity measured at 26°C in the casts (faecal casts) of 4 earthworm species cultured in paper waste sludge and 2.5% phytin. P.ex, *P. excavatus* Lm, *L. mauritii*, Pe, *P. elongata*; Pc, *P. corethrurus*; Raw food, contained paper waste sludge and phytin without worms (control).

Table 3. Faecal phosphatase activity in the casts of some earthworm species on paper waste sludge substrate for 6 weeks.

Culture residue	$\mu\text{g phenol g dry wt}^{-1} \text{ 3 h}^{-1}$	
	at pH 3/4	at pH 9/9.5
Wormless control (raw food)	776 \pm 21	182 \pm 16
<i>L. mauritii</i>	1251 \pm 82	1146 \pm 79
<i>P. elongata</i>	964 \pm 18	926 \pm 19
<i>P. excavatus</i>	1942 \pm 32	1753 \pm 38
<i>P. corethrurus</i>	838 \pm 21	712 \pm 15

Values are mean \pm SD of 5 samples.

Table 4. Effects of *P. excavatus* activity on the mineralization of paper sludge and garden soil in a period of 6 weeks.

Parameter	Paper sludge with phytin		Garden soil	
	Wormless control	Worm- activated	Wormless control	Worm- activated
pH change	6.8 \rightarrow 6.8	6.8 \rightarrow 6.5	7.2 \rightarrow 7.2	7.2 \rightarrow 8.0
Body weight growth (%)	—	-12.83	—	+2.38
Mortalities (%)	—	3-4	—	Nil
Pi ($\mu\text{g g dw}$)	189	242	486	689
Mineral content* of the residue (%)	43	52	35	58

*Ash content.

worm-activated garden soil, but the body weight-growth of the worm appeared to be retarded in cultures with paper sludge and phytin (table 4). The worm activities in soils caused a noticeable pH increase while in paper sludge cultures there was a considerable pH-drop (table 4). Worm cast deposition in soils probably may alkaline the soils. About 3 to 4% mortality was noted in paper sludge cultures in contrast to none in soil cultures (table 4). The phosphatase activity (at pH 9) of the paper sludge casts of *P. excavatus* showed a linear and direct increase as the concentration of phytin in the medium increased (figure 2). Phytin contains organic P and as the substrate concentration increased, the worms gut phosphatase activity increased.

A culture time of 3 weeks seemed to develop a significant faecal phosphatase activity. Furthermore, it was found that phosphatase activity in the paper waste casts of *P. excavatus* was dependent on temperature at which the culture was maintained as well as on the time of culture (figure 3). At higher temperatures the activity was found to be greater and stable, and with culturing time the activity exhibited a sigmoid curve (figure 3). Wormless controls exhibited poor activity at all temperatures and culture time. Three weeks old culture at any temperature seemed to produce casts with nearly 70-90% of total phosphatase activity (figure 3) obtainable by the culture. These results illustrate the extent of mineralization rates of P by the faecal phosphatases of worms which especially in tropical environments vary with season, and day and night periods.

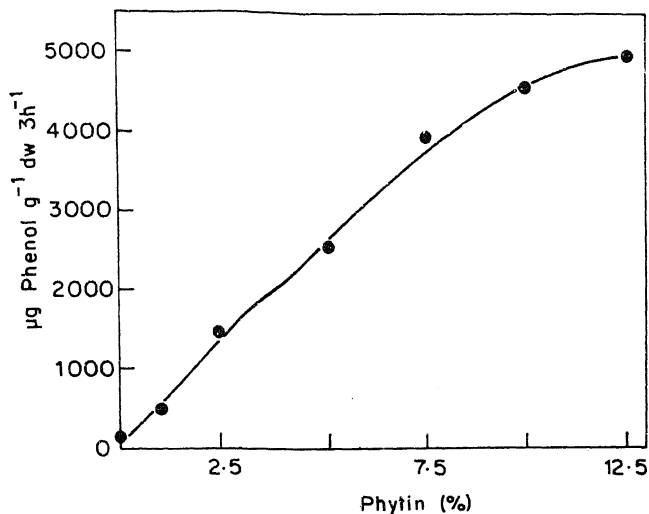


Figure 2. Phosphatase activity in the faecal casts of *P. excavatus* cultured for 4 weeks in paper waste sludge, at pH 8.5, temperature 25°C and varying phytin concentration.

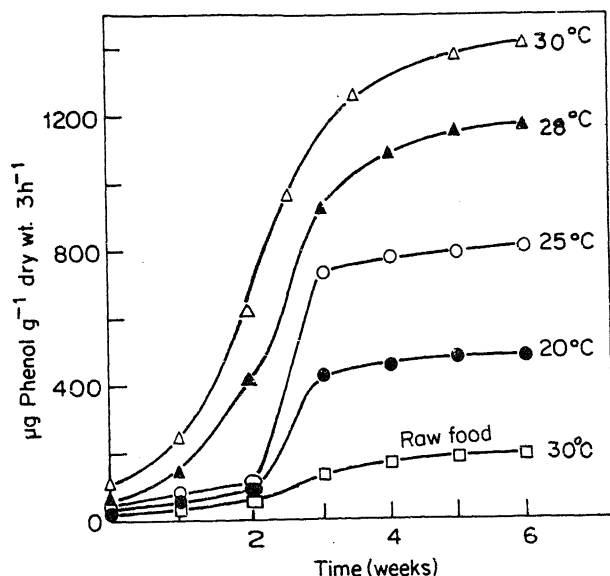


Figure 3. Development of phosphatase activity measured at weekly intervals and cultured in the casts of *P. excavatus* in paper waste sludge with 2.5% phytin in a BOD incubator set to the temperature shown in the figure and assayed at the same temperature at pH 8.5.

4. Discussion

The alkalinity of the cast soil observed here may be an indicator of beneficial effects of the worms in alkalinizing the soils. The wormcast soils in north-east India had shown significantly higher pH than the normal soil (Bhadauria and Ramakrishnan

1989). However Nijhawan and Kanwar (1952) noted a lower pH value for the worm cast soils of Punjab; which is in contrast with the present observations. The lowering of C/N ratios on casting observed in the present study however, corroborates the findings of Nijhawan and Kanwar (1952), but contradicts those of Shrikhande and Pathak (1948). Lowered C/N ratios in wormcasts however, indicate the existence of proportionately higher nitrogen available in the cast soil than in parent soil. Concentration of exchangeable elements like Na, Mg and K may be due to decomposition of organic matter, occurring during the passage of soil through worm's gut. Increase in Ca status of the cast may be due to calcium-oxalate-decomposing bacteria (Jakoby and Bhat 1958).

The enhancement of phosphatase activity in the worm-activated media described here are consistent with the observations of Graff (1970) and Sharpley and Syers (1976) on phosphorus mineralization by earthworm populations in field. The data are also consistent with the observations of Satchell and Martin (1984) on *Eisenia fetida*, and *Dendrobaena venata* in the laboratory. However, the present study suggests that among the species viz. *L. mauritii*, *P. corethrurus*, *P. elongata* and *P. excavatus* examined, the latter one bears greater potentialities to bringforth mineralization of organic P to inorganic P.

The wormcast phosphatase activity at pH 3–4 may be mainly a microbial activity (Satchell and Martin 1984). However, the possibility that the activity at pH 9–10 does not indicate the microbial activity cannot be excluded. However, in view of the many references existing on the functions of alkaline phosphatases in earthworms (Haase 1969; Dev and Vyas 1972) it seems more likely that these peaks indicate earthworm intestinal phosphatases. Overall, this study suggests that phosphatase activity in worm faeces may be substantially increased directly by the worm's own enzymes and indirectly by stimulation of the microflora present in the gut (Satchell *et al* 1984). The phosphatases in the wormcasts while their passage through the gut, start mineralizing the organic phosphorus into inorganic P which is the ultimate form of P required by the plants. The faecal phosphatases as evidenced in the present results, are fairly stable and more responsive to the temperature of the assay. Especially, in tropical conditions where soil temperatures are higher than those of sub-tropics, the P mineralization rates by the faecal phosphatases could be stable and higher.

The cast composition in the 4 species examined (table 1 and figure 1) varies widely. The increase in extractable inorganic P of 55% in the culture residues of *P. excavatus* seems somewhat greater than in those of other species (table 2), and the fact could be attributed to a higher level of mineralization occurring in the residue. This is confirmed by the increase in water soluble P which was about 80% in *P. excavatus*.

The presence of bacterial and faecal phosphatases in the wormcasts which are laid in soils may promote mineralization of soil organic phosphorus. This may be a minor role but essentially a direct metabolic reaction in the decomposition of plant material (Barley 1961). Based on the estimates of surface cast production in grasslands and woodlands around Bangalore (Krishnamoorthy 1985) and assuming that all those casts, are due to *P. excavatus* it is estimated that about 55 kg ha⁻¹ y⁻¹ inorganic P can be obtained in grasslands and about 38 kg ha⁻¹ y⁻¹ in woodland soils through mineralization. Out of these 19.79 kg of Pi ha⁻¹ y⁻¹ may be obtainable by wormcast phosphatase specific activity alone. The latter figure was

arrived at, by considering the activity of the faecal enzyme under the conditions prevailing in the field. These figures are obviously higher than the $9 \text{ kg Pi ha}^{-1} \text{ y}^{-1}$ reported for New Zealand pastures (Sharpley and Syers 1976). It must be emphasized, however that the Pi concentration in surface casts although higher than in underlying soil, is actually lower than that present in plant litter. For example, 59–83% of the total P in the litter from New Zealand pastures was estimated to be in the form of inorganic P (Gillingham *et al* 1976). By decomposing the plant litter in the soils, the surface casting earthworms thus help accelerating the rate of cycling of P in pasture and grassland situations.

The present results apparently also indicated that wormcasts have all major components that are essential (NPK) to regard them as field fertilizers. The data of table 1 particularly demonstrate that wormless parent soils (i.e. the controls) had a NPK ratio of 91:47:99 which on (worm) activation in the form of castings turned to 193:70:386 by *L. mauritii*, 189:68:391 by *P. elongata*, 208:140:381 by *P. excavatus* and 174:71:396 by *P. corethrurus*. These worm activations are comparatively better than those observed in the soils of north-east India (Bhadauria and Ramakrishnan 1989). Besides, the high urea and nitrogen contents relative to Pi content of the casts (table 1) obviously liken the status and significance of them as natural field fertilizers.

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Production of rotifer, *Brachionus plicatilis* Muller fed with different cell densities of microalgae, *Chlorogibba trochisciaeformis* Geiter

A S RAFIUDDIN and KUSUMA NEELAKANTAN

Department of Marine Biology, PG Centre, Karnatak University, Kodibag, Karwar 581 303, India

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Abstract. The production dynamics such as growth rate (K), doubling time (D_t) and production ($\text{ml}^{-1} \text{d}^{-1}$) on rotifer, *Brachionus plicatilis* was studied in different cell densities of microalgae, *Chlorogibba trochisciaeformis*. A significant increase in rotifer production was achieved at a density of 50×10^6 *Chlorogibba trochisciaeformis* cells ml^{-1} .

Keywords. *Brachionus plicatilis*; *Chlorogibba trochisciaeformis*; growth rate; doubling time.

1. Introduction

Several attempts have been made in recent years towards improving the culture conditions and nutritional quality of rotifers for aquaculture in view of their importance as the prime food in the initial stages of many crustacean and fish larvae. In addition to inert food (Hirata and Mori 1967), several species of algae have also been tried to determine their impact on growth of rotifers (James *et al* 1983; Lubzens 1987; Rezeq-Abu and James 1987). It was suggested that the monotypic algal feed had little effect on the reproductive rates of rotifers (Theilacker and McMaster 1971; Scot and Baynes 1978). On the other hand a substantial difference has been observed in reproductive rates of rotifers with different algal species (Snell *et al* 1983; Yufera *et al* 1983; Okauchi and Fukusho 1984).

In the present investigation, production dynamics of a rotifer, *Brachionus plicatilis* using different cell densities of a microalgae, *Chlorogibba trochisciaeformis* are dealt.

2. Materials and methods

The feeding experiments on rotifers was conducted using a local strain of marine microalgae a xanthophycean *C. trochisciaeformis* maintained in the laboratory at a temperature of 26°C , salinity 28 ‰ and pH 8.05. The algae were harvested in the exponential growth phase when the cell number was 70×10^6 and then diluted to obtain 10, 20, 30, 40, 50 and 60×10^6 cells ml^{-1} by adding seawater having a salinity of 28 ‰. The algal cells were counted with the help of a haemocytometer. The experiment was run in 4 replicates for each treatment using 5 litre beaker. Throughout the experimental period, the water temperature was maintained at $26.23 \pm 1.05^\circ\text{C}$, while providing continuous aeration.

The amictic rotifers numbering 1000 litre^{-1} were inoculated into the beakers and their population counts, pH and dissolved oxygen of the culture medium were monitored every 24 h. Each day 10% of the culture medium was filtered and the

corresponding volume was replaced with fresh seawater having desired cell densities of algae. The experiment was terminated after 7 days.

Doubling times were calculated by dividing $\log e^2$ by the instantaneous growth rate (K) from the expression

$$K = \frac{\ln N_t - \ln N_0}{t},$$

where N_0 =initial number of rotifer, N_t =final number of rotifer and t =duration.

2.1 Statistical analysis

The production ($\text{ml}^{-1} \text{d}^{-1}$), doubling time (Dt) and instantaneous growth rate (K) were subjected to test of significance by adopting the second degree polynomial regression formula (Snedecor and Cochran 1967).

3. Results

The results show that the production of rotifers per ml per day increased with increasing cell density of the feed (algae), but at higher cell concentration of the latter, the number of rotifers were found to decrease. At 50×10^6 concentration of algal cells ml^{-1} in the culture medium, maximum production of rotifers ($69.91 \pm 4.7 \text{ ml}^{-1} \text{d}^{-1}$) was discerned (table 1). The production of rotifers showed a linear relationship with the cell density of the algal feed (figure 1A). The increase in rotifer production between different treatments was significant ($P < 0.05$), but at higher algal cell concentration (50 and 60×10^6 cells ml^{-1}) the production of rotifers was insignificant ($P < 0.05$). The doubling time of rotifers decreased from 0.98 ± 0.02 to 0.81 ± 0.01 with increasing cell densities of the algal feed (figure 1B), which showed a significant difference among the treatments ($P < 0.05$).

The instantaneous growth rate (K) of rotifers showed a curvilinear relationship with the algal feed (figure 1B) where the values for the former increased from 0.71 ± 0.01 to 0.87 ± 0.01 with corresponding increase in cell densities of the latter (table 1), but whereas at a concentration of 60×10^6 algal cells ml^{-1} the growth rate ($0.86 \pm 0.01 K$) of rotifers was found to be less pronounced. However, a significant ($P < 0.05$) difference of growth rate (K) of rotifers was noticed between 10 and 60×10^6 cells ml^{-1} , reaching an asymptote stage beyond an algal densities of 40×10^6 cells ml^{-1} . The number of rotifers increased rapidly with increasing cell densities of algal feed. The mean rotifer densities recorded were 430.5, 490.5, 352, 281, 164 and 156 rotifers ml^{-1} at algal cell densities of 60, 50, 40, 30, 20 and 10×10^6 cells ml^{-1} respectively. The maximum density of rotifer increased between 5 and 6 days, whereas on other days the increase in population was not prominent.

4. Discussion

The increase in population density of rotifers with the corresponding increase of cell number of algae (*C. trochisciaeformis*) was apparent in the present study. Beyond an algal concentration of 50×10^6 cells ml^{-1} , there was a reduced growth rate of rotifers. The present results are in accordance with the findings of Rezeq-Abu and

Table 1. Production of rotifer, *B. plicatilis* in different cell densities of algal feed *C. trochisciaeformis*.

<i>C. trochisciaeformis</i> cell densities ($\times 10^6$ cells ml^{-1})	Repli- cates	Initial No. of rotifer ($\times 10^3$ ml^{-1}) (a)	Final No. of rotifer ($\times 10^3$ ml^{-1}) (b)	Duration (days)	Number of rotifer produced ($\times 10^5$ ml^{-1}) (b-a)	Rotifer production ($\text{ml}^{-1} \text{d}^{-1}$)	Doubling time (<i>Dr</i>)	Instantaneous growth (<i>K</i>)
10	1	5.2	7.7	7	7.65	21.851	0.98	0.71
10	2	5.3	8.5	7	8.45	24.134	22.24	0.98
10	3	5.5	6.9	7	6.85	19.557	\pm 1.00	\pm 0.69
10	4	5.6	8.25	7	8.20	23.411	1.75	0.71
20	1	5.7	8.7	7	8.64	24.694	0.96	0.72
20	2	5.6	6.5	7	6.44	18.411	23.27	0.68
20	3	5.1	9.3	7	9.25	26.425	\pm 0.94	\pm 0.74
20	4	5.4	8.3	7	8.25	23.56	2.99	0.72
30	1	5.3	13.95	7	13.90	39.705	0.87	0.80
30	2	5.5	11.60	7	11.55	32.987	40.03	0.87
30	3	5.2	14.70	7	14.65	41.851	\pm 0.86	\pm 0.81
30	4	5.2	16.00	7	15.95	45.567	4.57	0.82
40	1	5.3	18.00	7	17.95	51.279	0.84	0.83
40	2	5.5	15.00	7	14.95	42.701	50.13	0.85
40	3	5.9	19.00	7	18.94	54.119	\pm 0.84	\pm 0.83
40	4	5.9	18.40	7	18.34	52.403	4.40	0.82
50	1	5.6	25.50	7	25.44	72.697	0.80	0.87
50	2	5.7	22.30	7	22.24	63.551	69.91	0.81
50	3	5.9	26.60	7	26.54	75.833	\pm 0.80	\pm 0.87
50	4	5.4	23.70	7	23.65	67.559	4.70	0.87
60	1	5.5	22.50	7	22.45	64.128	0.81	0.86
60	2	5.6	23.60	7	23.54	67.270	62.20	0.81
60	3	5.7	21.20	7	21.14	60.41	\pm 0.81	\pm 0.85
60	4	5.3	20.00	7	19.95	56.991	3.87	0.85

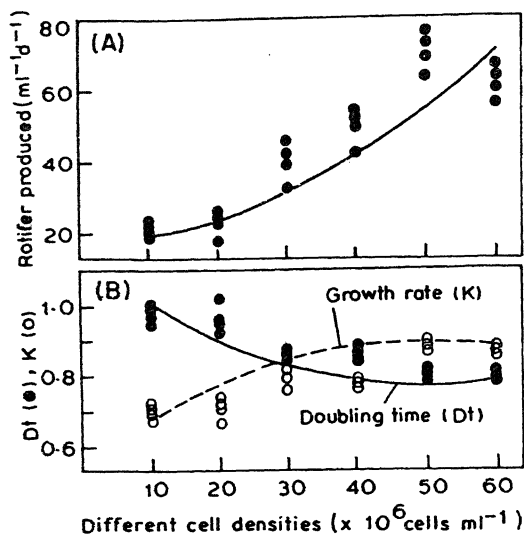


Figure 1. (A) Production of rotifers in different cell densities of *C. trochisciaeformis*. Each point represents the individual value of each replicate (rotifer production $\text{ml}^{-1} \text{d}^{-1}$ $Y = 1.12 + 1.229 X + 0.00033 X^2$; $r^2 = 0.85$, $n = 24$); (B) Instantaneous growth rate (K) and doubling time (Dt) of rotifers in different cell densities of *C. trochisciaeformis* (K , $Y = 0.7873 + 0.000195 X + 0.00000004 X^2$; $r^2 = 0.85$, $n = 24$, Dt , $Y = 0.8747 + 0.000163 X + 0.00000003 X^2$; $r^2 = 0.85$, $n = 24$).

James (1987), who concluded that, beyond a concentration of $(37.5 \times 10^6 \text{ cells ml}^{-1})$ of *Chlorella* sp, the rotifer population may reach a steady state without further increase in production. James and Rezeq-Abu (1988) have observed that the rotifer fed with an algae, *Chlorella capsulata* showed an increase in population density, production and growth rate only up to $10 \times 10^6 \text{ cells ml}^{-1}$ beyond which the population tended to decline.

Earlier investigation (Hirayama *et al* 1973) on the contrary with the present findings, has suggested that the most suitable cell density of *Chlorella* for optimum population growth and net reproduction rate of *Brachionus rubens* were $1.5 \times 10^6 \text{ cells ml}^{-1}$. Similarly Pilarska (1977) has also noticed the highest daily growth rate of *B. rubens* at cell densities of *C. vulgaris* ranging from 0.4×10^6 to $1.0 \times 10^6 \text{ cells ml}^{-1}$.

Yufra *et al* (1983) while using high cell densities (up to $100 \times 10^6 \text{ cells ml}^{-1}$) of marine algae, *Nannochloris* sp. and *Nannochloropsis* sp. for feeding two strains of *B. plicatilis*, observed an increase in density of rotifer at an optimum algal concentration of 50 and $70 \times 10^6 \text{ cells ml}^{-1}$. Earlier observations have suggested that rotifer could thrive on more than 2.6×10^6 *Chlorella* cells ml^{-1} , whereas, Rezeq-Abu and James (1987) have found more than 37.5×10^6 *Chlorella* cells ml^{-1} . In the present study a xanthophycean group of alga, *C. trochisciaeformis* was tested at different cell densities for the rotifer production. The results were similar to that of *Chlorella* sp. but there was a reduction in rotifer production beyond a concentration of $50 \times 10^6 \text{ cells ml}^{-1}$ of algae, *C. trochisciaeformis*.

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Colonization of marine foulants at a power plant site

N SASIKUMAR,* K V K NAIR and JAYAPPAUL AZARIAH

Department of Zoology, University of Madras, Guindy Campus, Madras 600 025, India

*Water and Steam Chemistry Laboratory, Applied Chemistry Division, Bhabha Atomic Research Centre, IGCAR Campus, Kalpakkam 603 102, India

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Abstract. Observations were made on the colonization of test panels (8 × 5 cm) by fouling organisms in the coastal waters and the cooling circuit (forebay) of Madras Atomic Power Station for a year. In coastal waters, hydroids settled as a pioneer species followed by barnacles and ascidians, whereas at the forebay, sea anemones were the important settlers. Species abundance, diversity indices and fouling biomass showed relatively low values at the forebay as compared to coastal waters. Species richness however was more in forebay than in coastal waters. Low species diversity with high species richness indices observed at the forebay could be attributed to species selection under altered environmental factors (chlorine levels, high flow rates and lack of predators).

Keywords. Power plant; fouling; succession.

1. Introduction

The settlement and growth of fouling organisms on surfaces associated with the power plant cooling water systems can interfere with the efficient operation of the plant (Hillman 1977). An evaluation of the type and abundance of such fouling communities from the cooling circuit of power stations provide information not only regarding efficiency of antifouling treatment adopted in such systems but also about the impacts of such antifoulants on the local ecosystem (Markowski 1959). Moreover, most of these organisms are sedentary or semisessile and thus are best indicators to assess any stress to the ecosystem.

Several studies from Indian coastal waters (Karande 1983; Satyanarayana Rao and Balaji 1987; Nair *et al* 1988; Sasikumar *et al* 1989) have reported very heavy fouling biomass build-up. Reports in the literature also indicate possibility of fouling biomass levels being different even in adjacent habitats. Most of the reports of the Indian authors have so far been on the development of fouling communities in natural habitats, there being very little data available on the fouling communities in impacted habitats. In the present paper, observations on the colonization of fouling organisms in a natural habitat (coastal waters) and inside the cooling circuit (forebay) of Madras Atomic Power Station (MAPS) are reported.

MAPS is located approximately 65 km south of Madras city, on the east coast of India. The power station uses seawater for cooling the condensers. Seawater is drawn through a 468 m long sub-seabed tunnel (figure 1) and is discharged through an outfall structure. Seawater enters the tunnel through subsurface intake gates (1 m depth) and reaches the forebay. From the forebay seawater is pumped at a rate of 35 m³/s for cooling the condensers. Chlorine is used as a biocide to reduce settlement of foulants in the cooling circuit. Chlorine dosing levels are maintained in such a way as to obtain a chlorine residual of 0.5 to 1 ppm at the forebay. The

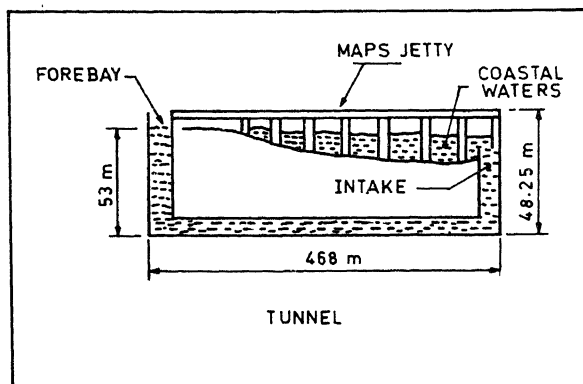


Figure 1. A schematic view of MAPS seawater tunnel.

forebay environment is characterized by the presence of chlorine residuals, high flow rates and absence of predators of common fouling life such as crabs, molluscs, echinoderms etc. and is thus quite different from coastal waters.

2. Materials and Methods

Twentyfour teak wood panels (each 8×5 cm) were suspended on mild steel frames from MAPS jetty as well as forebay at a depth of 1 m below the lowest low water of spring tides. One panel from each location was withdrawn every 15 days and macrofoulants were analysed quantitatively and qualitatively during 1986–87. A few panels from this series were lost after 250 days of immersion at the forebay. In addition, short-term panels were exposed for every 15 days at the same stations. Shannon Weaver diversity index (H) (Stirn 1981) and Menhinick's species richness index (d) (Odum 1969) were used to calculate the species diversity and richness respectively from the panel data. Following formulae were used to calculate indices.

$$\text{Diversity index } (H) = -\sum (ni/N \log ni/N), \quad (1)$$

where ni = importance value of the species and N = the total number of species.

$$\text{Richness index } (d) = S/(N)^{\frac{1}{2}}, \quad (2)$$

where S = importance value of the species and N = total importance.

3. Results

The data on seasonal variations in settlement of principal fouling organisms observed in the coastal waters and forebay are given in figure 2. In coastal waters (MAPS jetty), barnacles settled throughout the year, whereas at forebay, they were found only during February and August. At the forebay, hydroids settled from February to April with peak growth (50% coverage) in February. However, during other periods, they were totally absent on panels at this site. Though ascidians settled in coastal waters from April to August, they did not settle on panels immersed at the forebay. Sea anemones settled in large numbers from February to

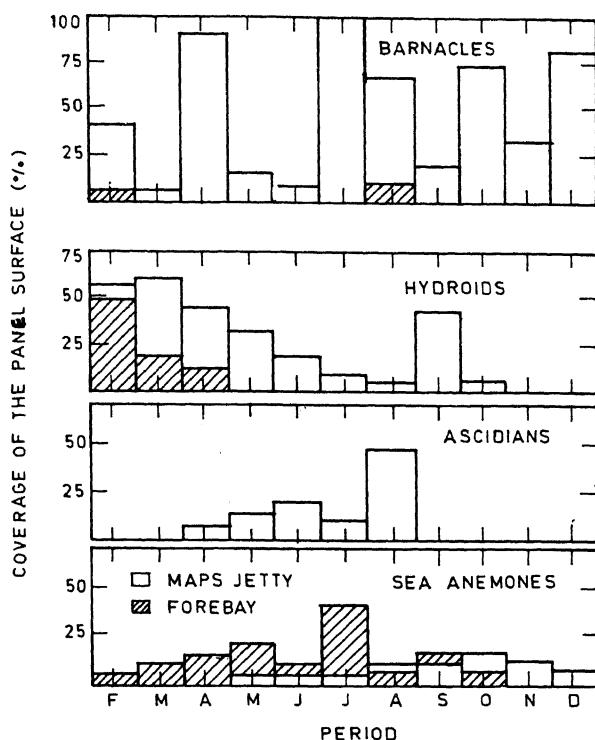


Figure 2. Seasonal settlement of principal fouling organisms in Kalpakkam coastal waters (MAPS jetty) and forebay; data collected from short term panels (15 days) at 1 m depth during 1986-87.

October at the forebay whereas settlement of this group was sparse in coastal waters except from September to December.

In coastal waters, fresh panels exposed for 15 days were completely covered with barnacles and hydroids (figure 3). After 50 days, hydroid colonies declined considerably and ascidians settled on the panels for the first time. Ascidians were the dominant group after 100 days. They covered most of the barnacles settled earlier. However, ascidian population dwindled after 200 days and disappeared altogether. Settlement of barnacles and sea anemones was also observed during this period. At the forebay, hydroids were the first to colonize and were abundant on 40-day old panels. Sea anemones settled after 40 days and covered 75% of the area in 125 days. However, sea anemone population declined after 160 days.

In the coastal waters, species diversity indices of foulants were characterized by 3 maxima (after 80, 200 and 275 days) and 3 minima (after 110, 225 and 300 days) (figure 4). Species richness indices showed two notable maxima, after 200 days (0.19) and 300 days (0.29) and 3 minima (after 100, 150 and 250 days) (figure 5).

Biomass values on short-term panels in coastal waters ranged from 9 g/100 cm² in March to 51 g/100 cm² in January (figure 6). Similar data on biomass in the forebay ranged from 0.5 g/100 cm² in October to 12.6 g/100 cm² in February. In coastal waters, long-term panels registered a maximum biomass of 65 g/100 cm²

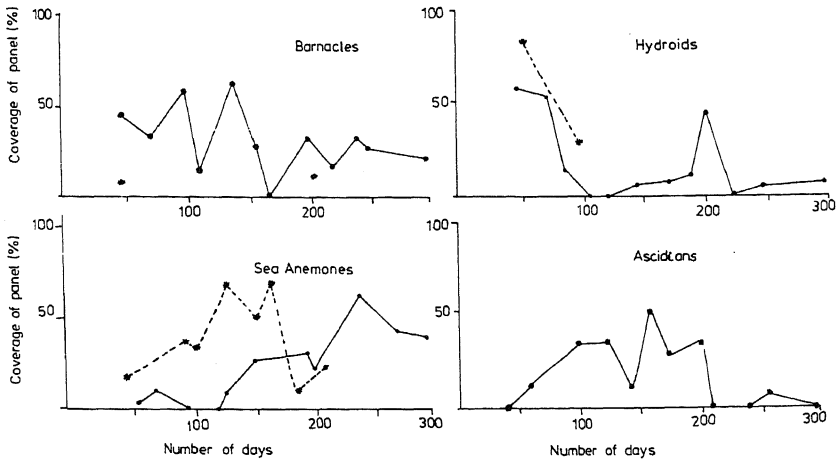
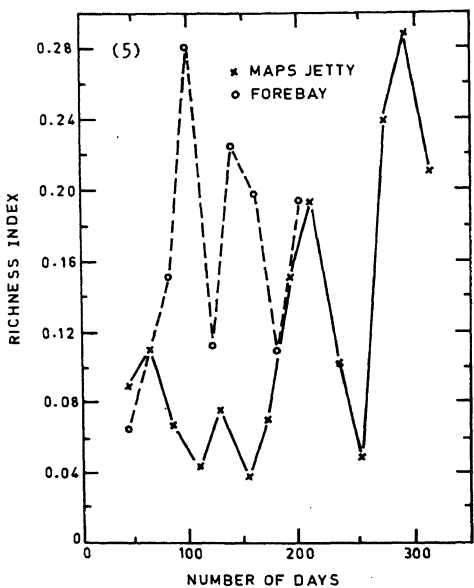
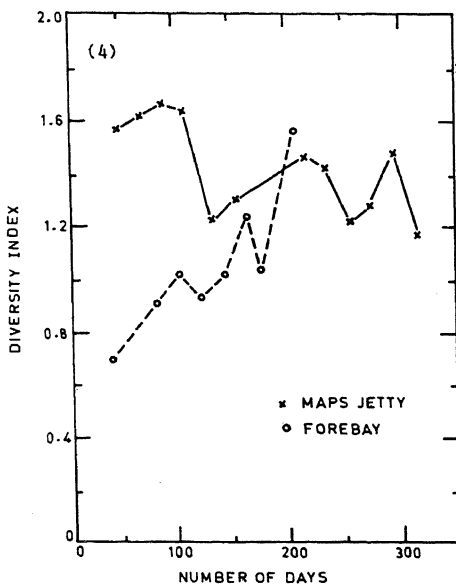


Figure 3. Settlement and colonization of principal fouling organisms on long term panels in coastal waters (MAPS jetty) and forebay during 1986-87. (●), MAPS jetty; (*), forebay.



Figures 4 and 5. Shannon Weaver diversity indices (H) (4) and richness indices (5) of fouled panels (long term series) exposed in coastal waters (MAPS jetty) and forebay during 1986-87.

(after 150 days) whereas maximum at the forebay was $13.3 \text{ g}/100 \text{ cm}^2$ after 170 days (figure 7).

4. Discussion

The study has helped to bring out the differences in the settlement pattern of

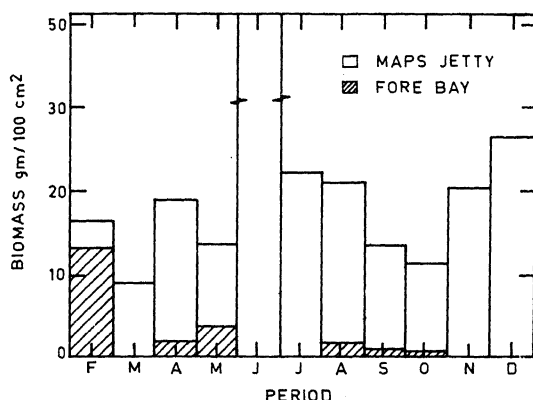


Figure 6. Seasonal variation in fouling biomass in coastal waters (MJ) and forebay (FB) during 1986-87. Data represent wet weight in g/100 cm² from short term panels.

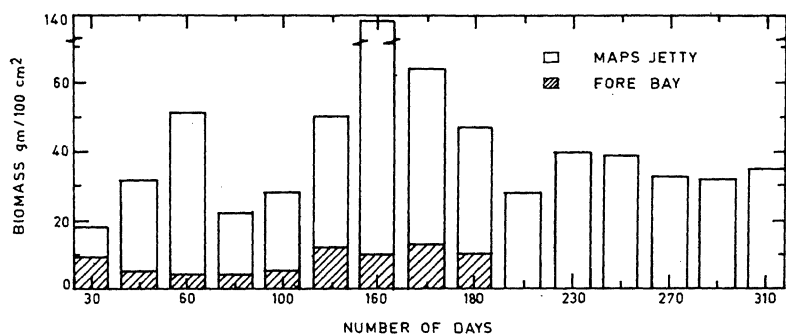


Figure 7. Cumulative biomass of foulants (long term series) in coastal waters (MJ) and forebay (FB) during 1986-87. Data represent wet weight in g/100 cm².

macrofoulants, their species diversity and biomass levels between coastal waters and forebay. While in coastal waters macrofoulants showed heavy settlement throughout the year, their settlement has been relatively low at forebay. Thus, barnacles and hydroids showed poor settlement in the forebay and ascidians, a group common in coastal waters were totally absent in the forebay. Sea anemones settled in larger numbers in forebay as compared to coastal waters. It looks reasonable to speculate that the presence of chlorine residuals, high flow rates and absence of predators could have made the forebay a substantially different environment from the coastal waters leading to the development of a different fouling assemblage. Such differences in colonization pattern between adjacent sites has also been observed by Sutherland (1981).

In a natural habitat, development of a fouling community is influenced by seasonal variations in larval recruitment, competition by dominant species and frequency of disturbance by other forces including predation (Sutherland 1981). During the process of colonization, these factors may influence abundance of species and often lead to total disappearance of many species. Such selection pressures eventually lead to the dominance of a better adapted competitively

superior species. In open coastal waters, such dominance by superior species appears to be a periodic phenomenon and no single species dominates the community for a very long time. This is mainly due to the frequent variations in larval abundance and settlement in open coastal waters (Raymond 1983). Under such conditions, heavy settlement of a species can eliminate a 'climax species' (Odum 1969). Sutherland (1981) suggested the term 'stable point' to describe the succession of fouling organisms and preferred this expression to the term 'climax' in this context. Such stable points can also contribute to heavy biomass build-up on a surface. Development of such heavy fouling biomass on harbour structures has been reported from Bombay (Karande 1968) and Visakhapatnam (Satyanarayana Rao and Balaji 1987) harbours, wherein *Mytilopsis sallei* has been the dominant species. In the present instance in coastal waters, the coverage of asidians on the panel has resulted in total disappearance of barnacles and other fouling organisms. However, ascidians dropped off from the panels after a while. This was followed by settlement of barnacles and hydroids once again. Thus, settlement and colonization of ascidians has been a major event in the succession of the fouling community in this area. Ascidians can therefore be considered as a temporary 'stable point' in the fouling community development in the coastal waters. However, such a stable point was absent at the fore-bay. Moreover, sea anemones, a major settler in the fore-bay appear to have had no significant influence on the nature of fouling community development.

Species diversity indices of foulants from the test panels showed relatively low values at forebay as compared to coastal waters. However, indices of species richness was high at forebay. Sutherland (1981) observed a decline in number of species in a polluted habitat at Puget Sound as compared to an unpolluted site. It appears reasonable to presume that such low species diversity and high species richness at forebay could be a result of selection pressures at this location.

At the forebay, seasonal and cumulative biomass data also showed low values as compared to coastal waters. Maximum fouling biomass observed at the forebay was approximately 10 times lower than that of coastal waters. Relini (1984) also reported similar differences in fouling biomass due to poor settlement at the outfall of Torvaldaliga Power Station.

Thus, species diversity, biomass and colonization pattern of macrofoulants showed significant differences between forebay and coastal waters. Further studies on the influence of selection pressures on community development in impacted environments are desirable.

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Human evolution from the Miocene to the Present

SHAMA BARNABAS

Ahmednagar College, Ahmednagar 414 001 and Division of Biochemical Sciences,
National Chemical Laboratory, Pune 411 008, India

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Abstract. Based on morphological, palaeoanthropological and molecular biological studies, human evolution from the Miocene to the Present has been reviewed. The initial divergence of orangutan from the hominoid stock, the divergence of man from the last common ancestor with the African apes, the origin and expansion of *Homo* lineage and the advent of modern man have been discussed.

Keywords. Human evolution; palaeoanthropological/molecular evidences; hominoid divergence; australopithecines; *Homo sapiens* origin; advent of modern man.

1. Introduction

The evolution of modern man (*Homo sapiens sapiens*) from his primate ancestors has generated great interest in recent years, due to new insights gained from palaeoanthropological, molecular and genetic studies. Discovery of a number of hominoid and hominid fossils in the recent past has added greatly to our understanding of human evolutionary history whereas molecular biology has provided quantitative information about the *Homo*/apes divergence as well as interrelationships within the human populations.

2. Hominoid radiation

I will trace the human evolution from Miocene [25–5.5 million years (myr)] times, during which, there was an extensive radiation of hominoids as is evidenced from the hominoid fossils found in different parts of the world. Some examples are, the early Miocene (16–18 myr) African apes like *Afropithecus*, *Turkanopithecus*, *Kenyapithecus* (Leakey and Leakey 1986a,b) and the Proconsul species (Clark and Leakey 1951; Walker *et al* 1986); the middle Miocene African apes from Fort Ternan, Majiwa, Buluk and Moroto (Pilbeam 1982; Raza *et al* 1983) and the late Miocene apes from Salonika and Macedonia in Greece, Yassioren in Turkey and Lufeng in China (Pilbeam 1982; de Bonis *et al* 1990). Asian hominoids including the ones from Siwaliks of India and Pakistan, have been included in the 8–12 myr old complex of *Sivapithecus* species. *Ramapithecus* also from Siwaliks was earlier regarded as ancestral to *Homo*, but later found to be very similar to *Sivapithecus* in features like molar enamel thickness, dental proportions and wear patterns (Delson 1985a). *Sivapithecus* shares a number of derived features with orangutan. These include a smooth subnasal plane, continuous with the floor of the nasal cavity; very narrow interorbital distance; flattened zygomatic bone and enlarged zygomatic foramina; very small incisive foramina, slit like palatine foramina, thick enamel and pronounced size difference between I¹ and I² (Andrews 1982; Andrews and Cronin 1982; Schwartz 1984). Thus it is probable that a late Miocene *Sivapithecus* species

was ancestral to orangutan. It has been suggested that following the dispersal of African hominoids to Asia, there was a division into two hominoid lineages, the African lineage leading to *Pan*, *Gorilla* and *Homo* and the Asian lineage leading to *Pongo* (Raza *et al* 1983). The *Sivapithecus*/*Ramapithecus* fossils represent the middle to late Miocene radiation of Asian hominoids of which the only survivor is the orangutan.

2.1 *Human, chimpanzee and gorilla clade*

Specific relationships between the extinct and living hominoids are not clear, but a much clearer picture of the divergence and relationships between the living hominoids has emerged from molecular geneological analysis. Goodman (1963) through extensive immunological comparisons of primate sera had shown that African apes should be considered as taxonomically closer to humans than to orangutans. Sarich and Wilson (1967) applied molecular clock concept to immunological distances of hominoid albumins using rabbit antisera produced against them and suggested an age of 5 myr for the last common ancestor of humans and African apes. Today, with the help of appropriate techniques we can discern the phylogenetic history encoded in the genome of an organism. Information of molecular geneology derived by using techniques like micro-complement fixation, radioimmunoassay, electrophoresis, amino acid sequencing, nucleic acid hybridization, restriction endonuclease mapping and nucleotide sequencing, has given an idea of the sequence and timing of the divergence of living hominoid primates. By taking into consideration all lines of evidences, Andrews (1987) has suggested that there were 3 main cladogenic events in hominoid evolution; first the divergence of gibbon lineage, ~20 myr ago; second, the divergence of orangutan, ~15.5 myr ago and third, the divergence of man-African apes, ~7-9 myr ago.

The gibbons which are the first to branch out from the hominoid stock, form a well defined group. They are primitive in their cranial and dental characters but highly specialized in postcranial and cytogenetic characters as well as in their behaviour (Mai 1983; Marks 1983; Andrews 1985). The great apes: orangutan, gorilla, chimpanzee and man have been grouped together and their inter-relationships assessed on the basis of shared characters. Orangutan, gorilla and chimpanzee appear to share a number of features of skeleton, musculature and reproductive system (Kluge 1983) but these have been shown to be of doubtful phylogenetic significance, except for a few characters like the separation of the flexor tendons of the toes, a feature which does not appear to be related to arboreal habit (Andrews 1987). Schwartz (1984) made a case for a relationship between orangutan and man based on a list of uniquely shared characters. Some of these characters appear to be primitive retentions, others are doubtful (records of longest hair) while a few could be synapomorphous. These are: a basicranial position of foramen lacerum, rare and unkeratinized ischial callosities and delayed ossification of the epiphyses (Andrews 1987).

The grouping of gorilla, chimpanzee and man with orangutan as the nearest outgroup, is supported by a large body of evidence. They can be grouped on the basis of a number of synapomorphous morphological characters (cranial, postcranial, reproductive and soft tissue features); immunological and electrophoretic analysis of proteins; DNA-DNA hybridization data; uniquely shared

amino acid residues and shared DNA substitutions. Thus the gorilla, chimpanzee and man clade seems the most acceptable. Within this group though, there is no agreement regarding which of the two lineages shared a period of common ancestry, to the exclusion of the third.

Chimpanzee and gorilla share two phylogenetically significant derived features: the enamel pattern (Martin 1985) and the complex of characters associated with knuckle walking (Tuttle 1967). In chimpanzee and gorilla the enamel is similar and secondarily thinned in the same manner and at the same rate. Both these species exhibit knuckle walking, an unusual form of locomotion. Neither orangutan (which supports itself on the sides of the fists) nor the human ancestors exhibited any adaptation towards knuckle walking. Chimpanzee and gorilla share a complex of 10 characters of the elbow, wrist and hand, which are either adaptations for knuckle walking or stabilising these parts (Tuttle 1967) and appear to be homologous.

Morphological, blood group and chromosome studies do not support chimpanzee/man grouping and immunological and electrophoretic studies have not helped in resolving interrelationships between the African apes and humans. However, as Goodman *et al* (1983) have pointed out that amino acid sequence data hints at the possibility that chimpanzee is more closely related to humans than to gorilla. This is due to the fact that at position 23 of α -haemoglobin, glutamic acid is present in human, chimpanzee and pigmy chimpanzee as compared to aspartic acid at this position in New World monkey, gibbon, orangutan and gorilla. Similarly, γ -haemoglobin chain of gorilla as well as the γ -chains of old world monkey have arginine at position 104 whereas human and chimpanzee have lysine at this position.

A distance matrix study of 183 DNA/DNA hybridization values (Sibley and Ahlquist 1984) favoured the grouping of man and chimpanzee. Also an extensive study of a 7.1 kb region of the ψ η -globin gene revealed that man and chimpanzee shared 8 derived features whereas gorilla/man and gorilla/chimpanzee shared two and three derived features respectively (Miyamoto *et al* 1987). In a more recent study (Gonzalez *et al* 1990), sequences totalling 3500 bases from 28S rRNA gene and from one of the ribosomal internal transcribed spacers (IIS) from human, chimpanzee, gorilla and orangutan show that human and chimpanzee are the most closely related pair. Holmquist *et al* (1988) have critically examined molecular evidences for human-chimpanzee, human-gorilla and chimpanzee-gorilla clades and have concluded that the available data do not clearly resolve higher primate phylogeny. However sequence data from primate β globin-gene clusters has provided evidence for a human/chimpanzee clade that narrowly excludes gorilla (Koop *et al* 1989).

3. Australopithecines

The period between 14 and 4 myr ago is poorly represented in the fossil record (Bishop and Chapman 1970; Pickford 1975, 1983). There are very few hominid fossils available before 4 myr. It would have been a period of important evolutionary changes in the African hominoids. During the Plio-Pleistocene, about 4 myr ago, fossil hominids found in eastern and southern Africa, have been included in the genus *Australopithecus*. This includes about 6 African species, among which are included the ancestors of *Homo* and the distinct robust *Australopithecus* lineage (Delson 1987).

3.1 Early australopithecines

A significant fossil, *Australopithecus afarensis* was near the ancestry of other *Australopithecus* species. The earliest *A. afarensis*-like hominid is about 5 myr old and found at Baringo in Kenya (Hill 1985). The ancestry of *A. afarensis* though not well understood should lie in the Miocene hominoid radiation of Africa. The African sites of Hadar in Ethiopia (3.3–3.0 myr old) and Laetolil in Tanzania (~3.7 myr old) have yielded a large number of *A. afarensis* remains. A study of these fossils indicates that they possessed primitive dental and cranial features (Walker *et al* 1986). Teeth form the largest part of these fossils and their study indicates that the dentition seems to be intermediate between the hominids and the pongids (Johanson and White 1979). In the skull there is strong alveolar prognathism related to the curved roots of the incisors. The canine roots are large, associated with strong canine jugae and the dental arcades are narrow with straight sides, not parabolic. There are other primitive features like, strong muscle markings on the cranium exemplified by a compound temporal-nuchal crest; a tubular pongid-like, external auditory meatus, broad mandibular fossae, a very flat, shallow palate, a pneumatised squamous temporal, large mandibular rami and condyle. The femur morphology, like the position of the lesser trochanter, height of the greater trochanter, the obturator insertion and the obturator externus groove shows that they were habitual bipeds (Clark *et al* 1984). This is supported by an analysis of the knee joint and the innominate bone as well as an enlarged occipito-marginal venous sinus system (Falk and Conroy 1983), another adaptation for bipedalism. The enlarged occipito-marginal venous sinus system, which allowed the blood to flow to the vertebral or the internal jugular system, was developed in response to the increased demand on the vertebral venous plexus due to the assumption of bipedalism.

The evolution of bipedalism was a step of enormous significance. It helped in the development of manipulative hands which could make and use tools as well as by freeing the mouth from the functions of carrying things and procuring food, eventually led to the evolution of language (Leakey and Lewin 1977). Among the living hominoids, the gibbon is specialized for an arm swinging locomotion and the much larger orangutan is also arboreal though much less acrobatic than the gibbon. The gorilla and chimpanzee leave the trees and walk on the ground by the rather specialized knuckle walking. Whereas man and his ancestors evolved a habitual bipedal form of locomotion.

There was marked sexual dimorphism in *A. afarensis*. The female, for example, 'Lucy', a rather complete adult skeleton from Hadar, was 3.5–4 feet in height as compared to the larger, morphologically identical male fossils. The brain, though small and pongid-like, already showed a limited degree of cerebral organization reminiscent of the human pattern (Holloway 1983).

3.2 Later australopithecines

The later *Australopithecus* species are: the 'gracile' *A. africanus* (3.0–2.3 myr old) from south Africa and the 'robust' species which include *A. aethiopicus* (2.5 myr old) from Kenya; *A. robustus* and *A. crassidens* (1.9–1.6 myr old) from South Africa and the hyper-robust *A. boisei* (2.0–1.3 myr old) from eastern Africa (Delson 1986). The Swartkrans site in south Africa offers evidence that *A. robustus* and *Homo* coexisted at this time. Burnt bones found in Member 3 from Swartkrans are the earliest

evidence of the use of fire in the fossil record. It is not certain whether the fire was used for cooking, protection from predators or warmth and also whether it was *Homo* or *Australopithecus* or both who used it (Brain and Sillen 1988).

The robust australopithecines are characterized by distinctive dental and cranial features associated with a large masticatory apparatus. These led to adaptations to help in maximizing the vertical occlusal force and spread it across the well developed post canine dentition (Johanson and White 1979). The face was massively built and subnasal prognathism was reduced. Incisors and canines were small and the post canine dentition was large, with molarized premolars. The zygomatics were robust, anteriorly placed and the temporal fossae were large. Sagittal crests were prominent and anteriorly located. In the lower jaw there was heavy buttressing of the mandibular corpus and the mandibular rami were broad. The brain case was located relatively low to the face and the foramen magnum was forwardly placed (Walker *et al* 1986).

The development of a grasping hand with separately movable fingers was also a significant advance in primate evolution. This led to the precision grip in which the thumb and index finger meet at the tips to form a circle. This was possible due to the gradual evolution of thumb opposability during primate evolution. Though many monkeys and apes exhibit some kind of precision grip, in man it is fully developed and used for fine manipulative skills. Though adapted for precision grip, the human hand did not lose the general ability for power grasping and prehensibility. The hominid hands now freed from the function of locomotion were used for handling tools not only for protection but also for procuring food, for example, digging up roots with the help of sticks.

The hand bones of *A. robustus* indicate that the hand was very much 'hominized' (Susman 1988). *A. afarensis* which was more than 3 myr old, did not possess human-like thumb and fingers, but by about 1.8 myr ago, a human-like thumb and fingers had evolved in the hominids. *A. robustus* hand possessed the specifically human flexor pollicis longus muscle, human-like metacarpals, a broad apical tuft on the distal end of the phalanx and short straight fingers. These features indicate that *A. robustus* was capable of precision grasping and tool behaviour, whereas lack of ape-like curvatures in the proximal phalanges indicate reduced power grasping and climbing ability. On the other hand, the overall period of dental growth and development in *A. robustus* and *A. boisei* (*Paranthropus*) was short suggesting that the prolongation of childhood growth period was not found in these hominids (Beynon and Dean 1988).

The cranial remains of *A. africanus* were first discovered in Taung (the Taung child's skull) in South Africa (Dart 1925). The skull has recently been studied using computerized tomography (Conroy and Vannier 1987) which has made it possible to scan it in different planes. This study has revealed that the developing dentition of *A. africanus* was similar to that of a 3-4 years old pongid. As in the pongids, at the first molar eruption, the other permanent teeth had not developed any roots, whereas in humans at this stage, the incisors, canines and third premolar possess some root structure. Also a great ape-like pattern of eruption of the permanent incisors and first molars as well as retardation of canine eruption was observed in the Taung skull. The horizontal alignment of the developing permanent incisors was great ape-like, indicating a greater degree of subnasal alveolar prognathism compared to humans. The Taung skull was a mosaic and as has been pointed out by Dart (1925), it also possessed a number of hominid-like features. For example,

lack of prominent supra-orbital ridges, narrow interorbital region, a higher forehead, more delicately built maxillae and mandibles, circular shaped orbits, lack of diastema in the lower jaw, forwardly located foramen magnum and an expanded brain.

Among the various views regarding the relationships between the early hominid species, two hypotheses are now more acceptable (Delson 1987). Both consider *A. afarensis* to be close to the common ancestor. The first hypothesis implies that the robust australopithecines were polyphyletic and includes *A. aethiopicus* in *A. boisei*. It suggests that *A. africanus* has given rise to *A. robustus* only, and does not clarify the ancestry of *Homo*. Suggesting polyphyly in the robust australopithecines, this view ignores the many derived features shared by *A. robustus* and *A. boisei*. The more popular second hypothesis suggests that *A. aethiopicus* is the sister taxon or common ancestor of the later robust species while *A. africanus* is the sister taxon of *Homo*. The earliest *Homo* fossils appeared about 2 myr ago in eastern and southern Africa. The closeness in time between the last appearance of *A. africanus* and the first appearance of *Homo* in the fossil record as well as their morphological similarities are considered to be evidences for an ancestor-descendent relationship between the two. Whereas, those who object to this view, point out that too much closeness in time between the last occurring *A. africanus* and the early *Homo* fossils make this relationship improbable (Walker and Leakey 1978).

4. Origin and expansion of *Homo* lineage

The reasons why the australopithecine and the *Homo* lineages evolved only in Africa are not clear. Perhaps the ecological changes consisting of shrinking forests and increasing open woodlands and savannas, in Africa, were the right impetus for the changes which took place during the evolution of the hominids; and if such niches were present in other parts of the world, they were occupied by competing primates (Leakey and Lewin 1977).

The *Homo* and the robust australopithecine lineages coexisted and evolved side by side as is evidenced from their fossils in the Olduvai Gorge region in northern Tanzania and Swartkrans site in South Africa. The robust australopithecine lineage became extinct due to reasons which are not so clear whereas the *Homo* lineage led to the evolution of modern man.

The earliest *Homo* species is *Homo habilis*. It first appears in the fossil record between 2.2 and 1.8 myr ago (Johanson and White 1979; Hughes and Tobias 1977; Howell 1979). Olduvai Gorge has yielded a very large number of fossils belonging to *H. habilis* and *A. boisei*. Some other early *Homo* fossils from Africa are, those from Koobi Fora formation (Leakey 1976), Omo Shungura Members, E, F, G, Olduvai Bed 1 (Leakey *et al* 1964) and Sterkfontein member 5 (Hughes and Tobias 1977). A study of the Olduvai *H. habilis* fossils indicates that, it was a small hominid exhibiting a mosaic of primitive and derived characters and rudimentary tool making skills. The fore limb was shorter than the hind limb. With an opposable pollex, the hand was capable of not only a power grip but also a simple precision grip. The pollical carpometacarpal joint and the distal phalanges were very human-like and the middle phalanges were robust and curved with well defined flexor digitorum superficialis insertions suggesting retention of climbing ability which

perhaps gave it a selective advantage, being able to escape, feed and even sleep in the trees. In the foot, the plane of the first tarsometatarsal joint is human-like; the fossil hallux is stout with human-like proportions and shows less axial torsion than that of apes. The morphology of the foot and leg bones indicates that *H. habilis* possessed human-like bipedality (Susman and Stern 1982).

The mean cranial capacity was larger than that of the australopithecines. Chin was retreating and maxillae and mandibles were smaller. Incisors were larger and the premolars were narrower in comparison to the australopithecines. Canines were larger relative to the premolars and molar dimensions could be compared to the lower range molar dimensions of the australopithecines. The supraorbital part of the cranium shows variation from a massive supraorbital torus to a smooth brow region. Also an *Australopithecus*-like post-orbital constriction and a concave or dishd appearance of the facial region was lacking. The face varied from moderately prognathous to orthognathous (Leakey *et al* 1964).

Opinions vary regarding the homogeneity of various *H. habilis* fossils. Some regard them as belonging to a distinct though variable taxon, others regard them as a mixture of *A. africanus* and *Homo erectus* (Robinson 1965b) a form more advanced than the *H. habilis*. They are also considered to be composed of two *Homo* lineages one leading to *H. sapiens* and other to *H. erectus* (Leakey 1966), and still others suggest different splits of *H. habilis* (Day *et al* 1980). However, most workers agree that there is too much variation in the *H. habilis* fossils to be included in one taxon. Attempts to split *H. habilis* on the basis of size and shape differences (Wood 1985; Stringer 1986) lead to formation of two subgroups; one with large brains, relatively large teeth and orthognathic faces such as KNM-ER1470 and 1590, and the other with smaller brains and lightly built faces such as KNM-ER1813 and OH24 (Wood 1987). It is not certain whether these two morphs represent sexual dimorphism or different taxa.

The next hominid fossil on the line to *H. sapiens* is *H. erectus*, once called *Pithecanthropus erectus* or 'Java man' by Dubois, in 1894. It is well accepted that it is close to the forms who were directly ancestral to the earliest *H. sapiens*. The 1.2 and 0.25 myr old skulls of *H. erectus* have been collected from Java and China (Pope 1984; Rukang 1985). Older *H. erectus* fossils have been found in Africa, for example, the 1.6 myr old fossil from west Turkana in Kenya which preserves almost the whole skeleton (Brown *et al* 1985) and also post cranial fossils from Olduvai Gorge in Tanzania (Day 1971) and east Turkana, Kenya. Thus it appears that *H. erectus* groups perhaps in search of food or even out of curiosity travelled across the narrow bridge of land that joins the African and Asian continents, to enter the northern continents. They were mentally better equipped than their predecessors and could make and use tools.

The hominids of Olduvai Gorge region are responsible for two distinct stone cultures (perhaps produced by two groups or tribes); the simpler Oldowan culture characterized by crude choppers and scrapers and the more complex Acheulian culture characterized by the hand ax. This was a tear shaped stone tool with sharpened edges and must have required considerable skill in making. The Acheulian culture followed the simpler Oldowan culture but the two cultures also coexisted for a long time (Leakey and Lewin 1977). The hominids of east lake Turkana are responsible for yet another stone tool culture, the Karari industry which had stone tools resembling the developed Oldowan industry. In parts of

southeast Asia, India, China and Burma, there existed a pebble tool technology which resembled the developed Oldowan industry.

It has been questioned whether the Asian and African *H. erectus* belong to the same taxon (Delson 1985b). Some claim that the two share unique derived features (Rightmire 1985) whereas others feel that they only share conservatively retained features. It appears that the Asian *H. erectus* has not contributed any mitochondrial DNA lineages to the modern man's gene pool (Cann *et al* 1987). Thus most probably the Asian *H. erectus* was later replaced by *H. sapiens* migrating from Africa.

H. erectus exhibited considerable sexual dimorphism and was a habitual upright biped of good height. The study of *H. erectus* fossils from west Turkana, Kenya (KNM-WT-15000) (Brown *et al* 1985) indicates that though human-like in many respects, the vertebrae differed from those of *H. sapiens* in having longer spines, narrower laminae and smaller vertebral foramen in cervical and thoracic vertebrae. The birth canal diameters were smaller than those of *H. sapiens*. A well preserved partial *H. erectus* innominate from Plio-Pleistocene in Koobi Fora formation, Kenya (Leakey 1976) had a similar position and orientation of the ischial tuberosity among other morphological similarities with that of *H. sapiens*. The relatively complete cranium of *H. erectus*, from Koobi Fora formation, is large in size, with thick vault bones. The supraorbital tori are prominent and post orbital constriction is reduced. It has a marked postglabellar sulcus, strong temporal lines and small temporal fossae. The zygomatic region is deep and wide (Leakey and Walker 1976).

Fossils like Laetoli Hominid 18 (LH 18) (Day *et al* 1980) from Tanzania, which is regarded as an early east African *H. sapiens*, give us a glimpse of the transition from *H. erectus* to *H. sapiens*. The LH 18 is about 120,000 years old and shows a mixture of advanced (expanded skull, vertical parietals, rounded occiput and cranial capacity of 1200 cm³) and primitive features (large brow ridges, low sloping frontals, marked thickness of bones). Such a mixture of characters is seen in other fossils also, for example, Omo skulls from Kibish Beds in Ethiopia (~130,000 years old) (Day 1969), Broken hill skull from Zambia (110,000 years old) (Bada *et al* 1974), skulls from Saldanha in South Africa, Bodo in Ethiopia and Ndutu in Tanzania (Kennedy 1980) range from the more primitive and *H. erectus*-like (skulls from Bodo and Ndutu) to the more advanced and *H. sapiens*-like (LH 18 and Omo skulls). A mixture of primitive and advanced features is also observed in post-cranial skeleton such as the femora from the Broken Hill locality and Guomde Formation east of lake Turkana. Opinions vary regarding the change in *H. erectus* during its evolutionary history from 1.6 myr to the middle Pleistocene (Delson 1985b), some advocating gradualistic change, others, a definite change over a period of time.

5. Advent of modern man

The *H. sapiens* includes two subspecies, the anatomically modern man (*H. s. sapiens*) and the neanderthal man (*Homo sapiens neanderthalensis*). The origin of anatomically modern man has been a hot topic of debate recently due to new information gained from palaeontology and molecular biology. The *H. s. sapiens* are characterized by derived features such as, a gracile skeleton with comparatively thin cranial and mandibular bones, a large, high and domed cranium, reduced or lacking

supraorbital torus and external cranial buttressing, reduced dentition and supporting parts, resulting in an orthognathous face (Stringer *et al* 1984).

The *Homo* lineage was not only characterized by definite anatomical features, but related to these were social and behavioural changes. *Homo* established home bases and collected and shared food. The food was mostly plant in nature but also included small animals. The food gathering habit was replaced to some extent by hunting. It has been suggested that the role of organized food hunting in human evolution has been exaggerated and that the habit of food sharing in organized social groups played a more important role in human evolution (Leakey and Lewin 1977). *Homo* was able to use his hands not only for carrying food but also perhaps water. This would have helped him greatly in his migrations which would have taken him away from water sources. *Homo* also learnt how to use fire perhaps after observing natural bush fires. He may have used it for warmth, light and protection and later also for cooking. A reduction in the size of the teeth and jaws is evident in the *Homo* lineage. Another significant character the *Homo* lineage and the other higher primates inherited from their ancestors was stereoscopic colour vision, enabling them to study their surroundings in colour and in 3-dimensions.

The neanderthal man lived between 30,000 and 100,000 years ago and was so named because of the discovery of its remains in Neanderthal valley in Germany. His remains have been found in different parts of Europe (for example, Swanscombe in England, Biache in France, Ehringsdorf in Germany) and southwest Asia, in the late Pleistocene caves of Tabun, Amud and Kebara in Israel and Shanidar in Iraq. A rich collection of tools has been found together with the fossils and the differences between the tool assemblies indicate that perhaps there were about 4 coexisting neanderthal cultures (Leakey and Lewin 1977). Unlike the popular opinion of the neanderthals as being brutish men with stocky, stooping muscular bodies, they appear to have had a complex culture. In the Shanidar cave, of the Zagros mountain highlands of Iraq, a 60,000 years old neanderthal burial site was discovered. A study of the pollen showed that several different species of flowers, some of them of medicinal value, were arranged around the body. Thus the neanderthals not only showed very human-like behaviour in burying their dead, but had also begun understanding and using some of the plants around them.

A study of the neanderthal fossils shows that they possessed very large cranial capacity, large brows and protruding occipital region, rounded parietals, and curved robust long bones. An examination of an almost complete pelvis from the Kebara cave in Israel showed that the outlet was not bigger but of a different shape from that of modern man. Also the structure and orientation of the sockets into which the femur fits were quite different from that of modern man perhaps implying differences related to locomotion and posture between the two (Rak and Arensburg 1987).

The relationship between the neanderthal and the early modern man has been a source of controversy (Trinkaus 1984). There have been two opposing views. According to one, the neanderthals were ancestral to the modern man. The second suggests that the neanderthals were a distinct branch of the *Homo* stock that became extinct and has not contributed to the modern *H. sapiens* lineage; and the modern man, who arrived earlier than the neanderthals in the Mediterranean Levant, coexisted with them in this region. The *Homo* fossils which were found in the southwest Asia late Pleistocene caves were earlier regarded as belonging to a

single population which was related to both the neanderthals and the early modern man. A more detailed study showed that the Tabun fossils were distinct neanderthals and were older than the Skhul samples which were early modern *H. sapiens*. It was assumed by many workers that just as in Europe, modern man appeared later than the neanderthal man in the whole southwest Asia also. The thermoluminescence dating of burnt Mousterian flints from Qafzeh caves in Israel has found them to be about 92,000 years old (Valladas *et al* 1988) which then also is the age of modern man in that region. Another modern man population at Djebel Irhoud in Morocco has been given a similar date (Delson 1988). Whereas, the neanderthal burial at Kebara (Israel) has been dated at ~60 kyr (Valladas *et al* 1987). Thus modern man was present in southwest Asia much earlier than the neanderthals and though the two would have evolved from a common ancestral gene pool, this observation makes an ancestor-descendent relationship between the neanderthal and modern man improbable. In fact the coexistence of the two in Eurasia for nearly 60,000 years, and lack of any definite evidence for a gene flow between them indicates that they may not be so close as to belong to the same species. The neanderthals appear to have been a *Homo* stock of European origin (they have not been found in Africa) who migrated to southwest Asia relatively late.

5.1 Two models for the origin of modern man

The two most popular models proposed to explain the origin of modern man are: the regional continuity model (multiregional origin) and the single origin model (recent African origin). According to the regional continuity model, there was an early and middle Pleistocene radiation of *H. erectus* from Africa, leading to the establishment of regional populations in other areas of the world through local differentiation. These populations maintaining gene flow then evolved into modern *H. sapiens* in different parts of the world. The single origin model suggests that there was a relatively recent common ancestral population, most probably in Africa, that possessed most of the anatomical features displayed by the modern humans. The modern man originated here, in the early part of the late Pleistocene. This was followed by a radiation from Africa to Eurasia of a founder population and development of modern regional characters outside Africa.

A study of the genetic and fossil data made by Stringer and Andrews (1988), supports the single origin model quite convincingly. Their theoretical predictions from the two models, regarding the regional variation also hold true for the single origin model. For example, according to the multiregional origin model, the interpopulation differences would be high, more pronounced between each peripheral area; the intrapopulation variation would be more pronounced in the central areas of human range and transitional fossils from the transition of local ancestral population to modern humans would be widespread. Also there would be no definite temporal pattern in the appearance of *H. s. sapiens* characters in the different regions of the world. The predictions according to the single origin model are quite different and are found to be true. The interpopulation differences are comparatively less and more pronounced between the African and non-African populations; the intrapopulation variation is the most among the Africans; transitional fossils are found only in Africa and the modern *H. sapiens* set of

characters appear first in Africa, then southwest Asia and later in the rest of the world.

The middle Pleistocene *Homo* fossils from different areas give an indication of its regional populations in that period. Early neanderthal fossils were found in Europe; in England, France and Germany (Stringer *et al* 1984). Late *H. erectus* fossils were found in the far east, in Zhoukoudian and Hexian in China and in Indonesia (Ngandong material) (Wolpoff *et al* 1984). In southern Africa at this time there were fossils such as, the skull from Broken hill (Zambia) which bears resemblance to the fossils in the north, like Bodo in Ethiopia and the pre-neanderthal or early neanderthal material from Europe (Stringer *et al* 1984; Brauer 1984). As has been mentioned earlier, the late *H. erectus* from Asia appears to be quite distinct from that in Europe and Africa, indicating a west-east division of the middle Pleistocene hominids before the divergence of the neanderthals (Stringer and Andrews 1988). This pattern of variation would not be supported by the multiregional model, which would indicate a closer relationship between the Eurasian populations.

The earliest (~100,000 years old) modern man fossils are from the south African caves of Klasies river mouth and Border cave (Stringer 1988), Skhul and Quafzeh caves from Israel, Dar-es-Saltane in Morocco and Omo-Kibish in Ethiopia. In Europe, the far east and Australasia, modern *H. sapiens* appears to have a later first appearance. Unlike what would be predicted by the multiregional model, there is a lack of morphological clines in Europe and Asia before the appearance of modern *H. sapiens*. There is absence of neanderthal derived features in the far east, Australasia and Africa (Santa Luca 1980; Stringer 1978) and not much evidence of a gene flow from other regions into Europe. With the appearance of modern man in Eurasia, there is the appearance of the African primitive and derived features in this region. Also inspite of a rather complete mid-Pleistocene fossil record, Europe and south west Asia lack neanderthal-modern *Homo* transitional fossils. On the other hand, the African fossil record presents intermediate fossils between the earlier *H. sapiens* and the modern man, for example, fossils from Omo-Kibish in Ethiopia, Ngaloba in Tanzania and Florisbad in South Africa (Stringer and Andrews 1988).

The early modern *H. sapiens* fossils from Africa and Eurasia show similarity in morphology as would be predicted from the recent African origin model. This is also true of some of east Asian and Australian early *H. sapiens* fossils (for example, the Mungo and Keilor fossils) which show similarities to the Eurasian fossils. However, some of the *Homo* fossils in the late Pleistocene and early Holocene of Australia show a very high degree of cranial variation. This can neither be explained by the multiregional nor the recent African origin model. It has been suggested by the supporters of the multiregional origin model that Australia may have been occupied by two founder populations (Wolpoff 1985), a gracile population from Asia represented by the Mungo and Keilor fossils and a robust population, derived from the Indonesian *H. erectus* and represented by the Kow Swamp and WLH-50 fossils. This would have given rise to a heterogeneity, which is not seen in modern Australians (Cann *et al* 1987). The recent African origin model holds true as far as the Mungo, Keilor and Niah cave (Borneo) fossils are concerned, which appear to be similar to the early *H. sapiens* from Eurasia. However, the more robust groups of Australasian fossils specially if they are as old as the gracile ones, are difficult to explain though it has been suggested that the more archaic looking Australian crania may be due to cultural practices of deformation (Delson 1988).

5.2 Molecular evidences for the origin of modern man

Molecular and genetic studies of modern populations support the idea of a sub-Saharan origin of modern man. Human mitochondrial DNA is a circular molecule, consisting of about 16,500 base pairs, is maternally inherited, does not undergo recombination and its mutation rate is about 10 times higher than that of nuclear DNA. These properties make it a very useful tool for the study of relationships. According to the multiorigin model one would expect to find marked genetic differences in mitochondrial DNAs between widely separated populations, but such differences have not been observed in the study of mitochondrial DNA of different geographic populations. Cann *et al* (1987) studied the mitochondrial DNAs of 147 individuals from 5 populations (sub-Saharan Africans, Asians, Caucasians, aboriginal Australians, and aboriginal New Guineans) by high resolution mapping with 12 restriction enzymes. They found 133 types of mitochondrial DNAs in the populations studied and made a phylogenetic tree, that relates the mitochondrial DNA types to each other and a derived ancestral mitochondrial type. The mitochondrial tree has two primary branches, one leading exclusively to Africans and the other leading to some Africans and to all other population groups. The evolutionary tree supports the idea of the African population being ancestral and this is also confirmed by the observation that Africans seem to have more mitochondrial DNA diversity than other populations. The variation within the African population was found to be as great as that between the Africans and other populations. The intrapopulation variation within the Asian group is comparable to that between populations whereas in the case of the Caucasians, Australians and New Guineans the within group variation is almost similar, the variation between groups being slightly more than that within the groups. A parsimony tree for 7 D-loop sequences of mitochondrial DNAs from Caucasians and black Americans, rooted by the midpoint method indicated that there was more diversity in the black D-loop sequences and the common ancestor was African in origin (Greenberg *et al* 1983). Cann *et al* (1987) also suggested that the mitochondrial DNAs are derived from a single woman (the mitochondrial eve), who lived about 200,000 years ago, indicating that the human population passed through a severe bottleneck. This timing is based on the assumption of 2-4% divergence in the mitochondrial DNA sequence per million years (Stoneking *et al* 1986). An objection to the mitochondrial eve hypothesis has been raised by Latorre *et al* (1986) who have studied the mitochondrial DNA types in *Drosophila subobscura* which has recently expanded into the new world, and have argued that one ancestral mitochondrial type can reach fixation by random genetic drift.

A study of the frequency of closely linked nuclear DNA polymorphisms (haplotypes) in the β -globin gene cluster, of normal chromosomes from Europeans, Indians, Asiatics and Africans also found that 3 β -globin haplotypes are common in all non-African populations and rare in Africa, whereas out of the two haplotypes which were common in the African populations, one was rare and the other completely absent from the non-African populations (Wainscoat *et al* 1986). The modern *H. sapiens* founder population which migrated from Africa into Eurasia appears to have lost the now predominant β -globin African haplotype which indicates that this founder population which gave rise to all the non-African populations was rather small (Jones and Rouhani 1986) and there was a population bottleneck at

this time in human evolution when the emigrant modern man left Africa and dispersed to the rest of the world.

A number of Negroid specific DNA polymorphisms have been found at loci such as α -globin, β -globin, growth hormone etc. (Chakravarti *et al* 1984), dihydrofolate reductase (Anagnou *et al* 1984) and insulin and albumin locus (Murray *et al* 1984). Thus the nuclear DNA data indicates a division between the African and Eurasian populations and also supports the recent African origin model. Studies of DNA sequences associated with sickle cell mutants, as well as blood groups and enzyme alleles data of African populations also suggests greater diversity indicating a longer history of human evolution in this region compared to other parts of the world (Pagnier *et al* 1984). Thus it appears that the combined molecular studies of autosomal nuclear DNA, mitochondrial DNA and Y-chromosome-specific DNA markers which would help in studying gene flow through the males, offer great potential for the genetic analysis of human populations.

5.3 Expansion of modern man

The 90,000 to 100,000 years old fossils of modern man from Israel and Morocco perhaps represent an ancestral Eurasian population which later entered the northern hemisphere. However, modern human fossils have not been found in western Europe before 35,000 years ago (Delson 1988). Thus the entry of modern man to south western Europe occurred around 35,000 years ago and slightly earlier in eastern Europe (Howell 1984). The 30,000 years old Cro-Magnon people of south-western France have always fascinated pre-historians with their sophisticated stone tool technology, carvings and cave paintings. The delay in modern man's further movement into the northern hemisphere may have been due to an unsuitable environment in the northern parts or competition with the well adapted and well entrenched neanderthals. It is also very likely that early modern human fossils have yet to be discovered in Eurasia. The first entry of modern man to Australia took place 40,000 to 50,000 years ago (Jones 1987; Roberts *et al* 1990) and the colonization of New Guinea took place from Australia about 30,000 years ago (Hope *et al* 1983). There is a controversy regarding the age of the first human colonization of the Americans. Two possible dates for the entry of modern man to America are ~ 35 kyr and ~ 15 kyr (Fagan 1987; Bray 1988; Dillehay and Collins 1988) with more consensus for the second date.

Thus there appears to have been an expansion of modern humans from Africa to the whole earth resulting in the replacement of the neanderthals and other archaic humans in different regions. The modern human populations appear to have expanded rather quickly to occupy the different areas of the world. Fully developed language could have been the stimulus for this kind of expansion (Cavalli-Sforza *et al* 1988). It evolved slowly over millions of years and reached its peak in modern humans, resulting in efficient communication and spread of information. Based on studies of laryngeal/basicranial morphology (Laitman 1985), it has been suggested that the neanderthals were incapable of language. Thus language could have given the modern humans an advantage over the neanderthals. Recently a well preserved human hyoid bone ($\sim 60,000$ years old) has been discovered from Kebara cave, in Israel (Arensburg *et al* 1989). It is found to be almost identical in size and shape to the hyoid of present day populations, thus implying that the morphological basis

for the development of language/speech was well developed in the neanderthals. There is not much evidence for the modern man having been directly responsible for the extinction of neanderthals. They could obviously not compete with modern man who was perhaps more progressive. They may also have been specialized for survival in very cold conditions and were not able to adapt to a change in the climate at the end of the last ice age.

The morphological variation shown by the present day populations is a result of adaptations to local environments and geographical separation. The stocky Eskimos from the northern regions are adapted for conserving heat while the very tall tribal Masaai from Kenya are adapted for losing heat. Variation in skin colour in the different populations is another adaptation; the melanin pigmentation in the human populations increases as one moves towards the equator because the need to protect the skin from ultraviolet radiation also increases.

From the above one can conclude that all the human populations, inspite of their morphological variations belong to one recently evolved family.

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Announcement of Merger

The Council of the Indian Academy of Sciences has decided to merge three of the biology journals published by the Academy: *Proceedings: Animal Sciences*, *Proceedings: Plant Sciences* and the *Journal of Biosciences*. Consequently, this will be the **last issue** of the *Proceedings: Animal Sciences*. For various practical reasons, it has been decided to retain the name *Journal of Biosciences* for the merged journal. This will be a quarterly and the first two issues combined will appear in June 1991. Since this is an important decision reversing an earlier one taken in 1978 it is appropriate to briefly mention some of the reasons underlying this merger.

Since the Academy was founded in 1934, its main activity has been the publication of scientific journals. The *Proceedings of the Indian Academy of Sciences* appeared in two sections: Section A was devoted to Physical Sciences and Section B to Life Sciences. In 1978, following the worldwide trend, the *Proceedings* were split into several subjectwise journals. In particular, Section B was split into *Proceedings: Animal Sciences*, *Proceedings: Plant Sciences* and *Proceedings: Experimental Biology*. The section on Experimental Biology was renamed the *Journal of Biosciences* in 1979. At first sight, it seems like a retrograde step to merge these three journals once again, particularly in the light of the present fashion and preference to have more and more specialized journals. But the main motivation for the rethinking is the following. It is well-known that the overwhelming majority of the better papers published by Indian scientists find their way into journals published abroad. Given this trend it has been particularly difficult to maintain high standards with **four** specialized journals in Biology (with 19 issues per year between them). Thus, sadly, the earlier hope that specialized journals are more likely to attract good papers than the *Proceedings* (which covered all of biology) has not been fulfilled. In the ultimate analysis, however, our priority is to publish papers of high quality. Indeed, in several editorials published in *Current Science* before the Academy was founded, C V Raman argued that the main objective of the Academy would be to publish journals where the more important results of the Indian scientists would appear, rather than be exported. Only then, he argued, can the Indian scientific community gain an international recognition and be freed from a position of semi-dependence. Seized with the problem of the urgent need to revitalize our journals, the Council of the Academy initiated several discussion meetings. The eventual decision to merge these three journals emerged from these meetings between several active biologists and the concerned Editors. Since the *Journal of Genetics* had a very different historical origin, it was felt that it should continue as an independent journal with a somewhat different perspective and character.

The new *Journal of Biosciences* will, in a sense, have a broader scope than the three separate journals that are being merged and will include all areas of biology such as Molecular Biology, Genetics, Developmental Biology, Biophysics, Biochemistry, Immunology, Endocrinology, Medical Biology, Neurobiology, Ecology, Physiology, Ethology, Evolutionary Biology, Environmental Biology, Sensory Biology etc. Consequently a new Editorial Board is being constituted

Announcement of Merger

whose composition will reflect this enlarged scope. This reconstitution will also give an opportunity to a new group of people to share in this important responsibility.

On behalf of the Council and the Fellowship of the Academy I wish to profusely thank all the members of the outgoing Editorial Board for their commitment and dedication to this journal during difficult times. Very special thanks are due to Prof. T N Ananthakrishnan for shouldering the responsibility of Editing this journal for more than a decade and for his untiring efforts to sustain this journal.

G Srinivasan
Editor of Publications